

Section II

Biochemical Screening and Structure-based Drug Design to Discover Antiviral Agents

CHAPTER 6

HIV Integrase Inhibitors

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6.1 Introduction

Progress in the discovery and development of human immunodeficiency virus (HIV) treatment therapy has been among the most significant accomplishments of medicine in the past quarter century and, arguably, a miracle of science. As a result, HIV has become a manageable disease under a strict set of conditions dependent on excellent medicines used in the right combinations along with obligations for the patient to comply closely with specific dosing guidelines. However, success does not occur in a vacuum and the situation is still dire for many who either do not have access to the right drugs for their profile or have complicated situations where optimal treatment is not possible. The most recent numbers for 2011 actually show an increasing number of HIV-infected persons, primarily as a result of a decrease in the number of deaths combined with a stabilized rate of new infections.¹ The numbers are still daunting: 1.7 million deaths annually, 2.5 million new infections and 34 million people currently living with the virus. Additionally, what was once primarily a male-centered disease is now increasingly affecting women, in particular women of color in the United States are disproportionately affected by HIV infection. While the therapy regimens in current use are significantly improved over the

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early days of the disease, it has become strikingly evident that the most basic part of the equation – getting patients to take their drugs – is a major reason for a lack of efficacy.^{2,3} Although patient behavior seems far removed from the discovery laboratory, the field has advanced sufficiently that building the appropriate properties into new antiretroviral drugs to allow for optimal patient convenience is now recognized as equally important to these drugs' virological properties.

HIV virus is relentless and continually evolving under drug pressure to escape control and continue the infection.⁴ The transmission of such a resistant virus is of concern and currently a topic of intense debate.^{5–7} To complicate matters further, the long-term tolerability and safety of many of the available therapeutic successes are not optimal.⁸ This creates more challenges for patient compliance. Of particular interest is finding regimens that allow delay of the use of nucleoside analogs (nuc sparing) until later lines of therapy.^{9,10} Ironically, one of the more interesting rationales for continued introduction of antiretroviral agents comes from the curative approaches that are now being explored. It has been argued that intensification of current regimens will be required for complete suppression during transcriptional activation envisaged during attempts to purge latent reservoirs.¹¹ As such, there continues to be a need for new drugs either from new classes or from further study of existing targets.

The above arguments support a need for new antiretroviral agents, but ones that are optimized for patient needs and that provide clear benefit and differentiation over existing options. On the surface, the field would appear to be well served, with over 30 treatment options. However, on closer inspection, many of these drugs center on the same mechanisms of action and are sufficiently similar that they do not provide unique characteristics orthogonal to other drugs already available. The key challenge for contemporary HIV antiretroviral drug discovery efforts is to choose the right target and match a fully optimized inhibitor while taking into account patient needs.

6.1.1 HIV Integrase

HIV is a retrovirus from the lentivirus family. The virus encodes 15 proteins, of which only three (reverse transcriptase, integrase and protease) have enzymatic activity.¹² Reverse transcriptase (RT) and protease (PR) are responsible for the conversion of single-stranded viral RNA into double-stranded DNA and the proteolysis of the gag polyprotein, respectively. Both RT and PR have proven to be excellent drug targets, with several antiviral drugs having been available for both for many years. Targeting virally encoded proteins or enzymes has traditionally been viewed as desirable due to a higher likelihood for selectivity, since they are not derived from the host. A third virally encoded enzyme is HIV integrase (IN). Integrase is a 32 kDa protein that is responsible for the integration of reverse-transcribed viral double-stranded DNA (dsDNA) into host chromosomal DNA during the retroviral replication cycle. As a result of this unique retroviral step, integrase has become an attractive target for drug discovery. The IN enzyme consists of a 288 amino acid primary sequence

divided into N-terminal, C-terminal and catalytic core domains. The catalytic core domain, consisting of residues 51–212, contains a triad of carboxylate residues at positions Asp64, Asp116 and Glu152, which is known as a D,D,E motif and is highly conserved in the catalytic active sites of the polynucleotidyl transferases superfamily.^{13,14} The three acidic residues are essential to enzyme function and coordinate two metal ion cofactors, presumably Mg^{2+} , under physiological conditions.^{21,22} These active site metals catalyze phosphodiester bond breaking and formation during the integration of viral and host dsDNA.¹⁵ The biochemistry of the integration catalysis consists of two distinct mechanistic steps involving removal of a terminal dinucleotide from the respective 3' ends of the viral nucleic acid in the cytoplasm (3' processing, Stage B to C in Figure 6.1a) and nicking of the host DNA by the newly exposed 3'-hydroxyl groups in the cell nucleus (strand transfer, Stage D to E, Figure 6.1a).¹⁶ Host cell repair mechanisms serve to complete the process of removing the remaining nucleic acid flaps and fill in leftover gaps due to the DNA nicking event. It is the strand transfer step that has been most amenable to inhibition thus far and the target of the molecules discussed below. A short section at the end of this chapter focuses on a newer class of inhibitors that appear, at least in part, to invoke their antiviral effect due to inhibition of the integrase 3' processing step.

Early screening efforts resulted in the discovery of what have become known as the diketo acid integrase inhibitors (DKAs) and acid isostere-containing molecules by researchers at Merck¹⁷ and Shionogi.¹⁸ The series of chemotypes that were discovered proved seminal discoveries for much research that was to follow; however, their clinical utility was far from optimal. The subsequent structure–activity relationship (SAR) work that was done clearly established some key pharmacophore attributes that include a metal chelating motif and a hydrophobic tail. The so-called two-metal pharmacophore was set forth and has served for more than a decade as the basic model for integrase strand transfer inhibitor design.^{19–21} A representation of the model is shown in Figure 6.1b, coordinated to an early DKA-derived drug known as S-1360.^{15,22} Further generalization of the model for consideration of additional molecular design is indicated in Figure 6.1c, whereby the basic elements of metal-chelating and hydrophobic motifs are present. Careful biochemical characterization studies demonstrated that these chelating pharmacophore molecules selectively inhibit the strand transfer step and have little effect on the earlier 3' processing event.^{23,24} Again referring to Figure 6.1a, the binding of an inhibitor results in the 3' processed intermediate, shown in Stage C, proceeding to an active site–drug complex, indicated by Stage X in the diagram. This binding of the chelating drug to the metals in the active site prevents coordination of the host DNA phosphodiester and its eventual cleavage stabilization by the resulting metals.

Recent progress in crystallography studies reported by Cherepanov and co-workers,^{25–27} using the prototype foamy virus (PFV) intasome complex with known IN inhibitors, has served to validate the two-metal pharmacophore concept. Moreover, the stacking interaction of the hydrophobic group with a

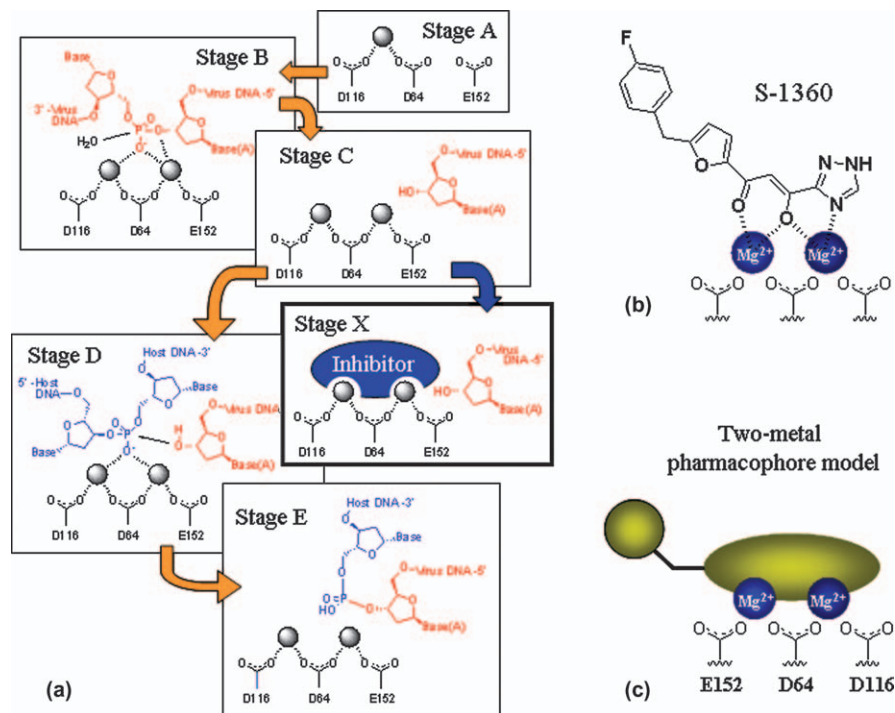


Figure 6.1 (a) A graphical scheme of a two-metal-ion catalysis and inhibition mechanism. Stage A to C indicates the 3'-processing reaction and Stage C to E the strand transfer reaction. Stage A to B: the enzyme recognizes the adenine base conserved in the third position from the 3' end of viral DNA, then activates the next phosphoric ester with the two metals. Stage B to C: the activated phosphoric ester is hydrolyzed to excise the terminal dinucleotide and the recognized adenosine is exposed as the new 3' end, giving a pre-integration complex. Stage C to D: the pre-integration complex non-specifically binds to host DNA to activate a phosphoryl ester by the two metals. Stage D to E: the activated phosphoric ester is attacked by the recessed 3' end in the manner of an S_N2 -like nucleophilic reaction, then the viral DNA and the host DNA are joined with each other. Stage X: an inhibitor chelates to the two metal ions of Stage C to block the host DNA binding. (b) S-1360 binding to the metal cofactors. (c) A graphical depiction of the two-metal pharmacophore model. Reproduced with permission from reference 20.

viral nucleic acid substrate base observed in the co-crystal structures helped explain some of the empirically determined minimal SAR requirements. Similar concepts of two-metal coordination have been employed to design inhibitors of the RNaseH motif of HIV-1 reverse transcriptase^{28,29} and an influenza endonuclease.³⁰

The observations described above stimulated extensive research efforts in this area over the past 15 years and have likewise met with considerable success.^{31–33} We begin our discussion where most of this work has culminated,

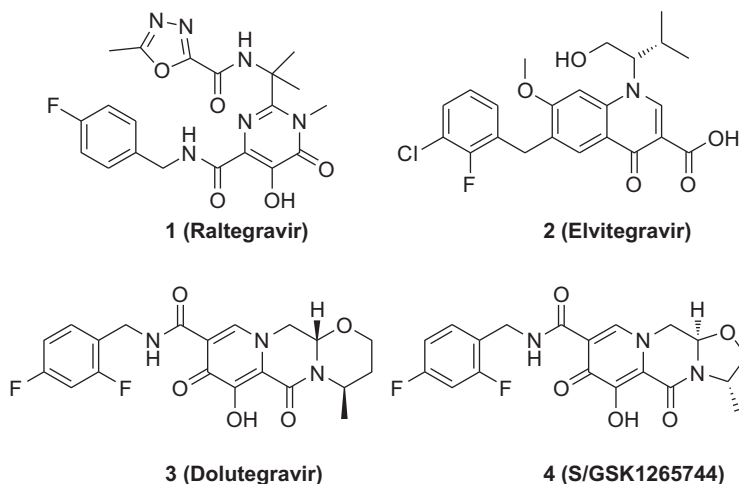


Figure 6.2 Structures of raltegravir, elvitegravir, dolutegravir and S/GSK1265744.

with a brief review of the discovery and development of two first-generation HIV integrase inhibitors (INIs), raltegravir and elvitegravir. The majority of our attention, however, will be centered on the discovery of a series of carbamoylpyridone-based ‘next-generation’ IN inhibitors, which led to the identification of dolutegravir (S/GSK1349572) and S/GSK1265744, currently under development for the treatment of HIV as oral and long-acting injectable agents, respectively (Figure 6.2).

6.2 First-generation HIV Integrase Drugs Raltegravir and Elvitegravir

As mentioned above, efforts to find a suitable molecule for clinical progression began in earnest after the DKA discoveries in the late 1990s. Several drug candidates progressed into clinical trials after extensive preclinical SAR and safety studies. These include S-1360,^{15,22} L-870810,³⁴ GSK364735,³⁵ GS-9160³⁶ and BMS-707035,³⁷ which were subsequently terminated as a result of insufficient pharmacokinetics (PK) to be either efficacious or differentiated from the competition or because of safety assessment findings that precluded further development. In 2007, however, after a host of failures, raltegravir (RAL) became the first INI to achieve approval and registration successfully. The discovery of raltegravir has been well documented by Rowley³⁸ and will not extensively be repeated here. To summarize (Figure 6.3), the key lead **5** came from a hepatitis C virus (HCV) program for inhibitors of the NS5B RNA-dependent RNA polymerase. This compound did not inhibit HIV integration, but the metal-binding capacity of the scaffold gave researchers at the Istituto di Ricerche di Biologia Molecolare (IRBM, a Merck subsidiary) the impetus to build in the hydrophobic benzylamide (**6**) and they observed remarkable

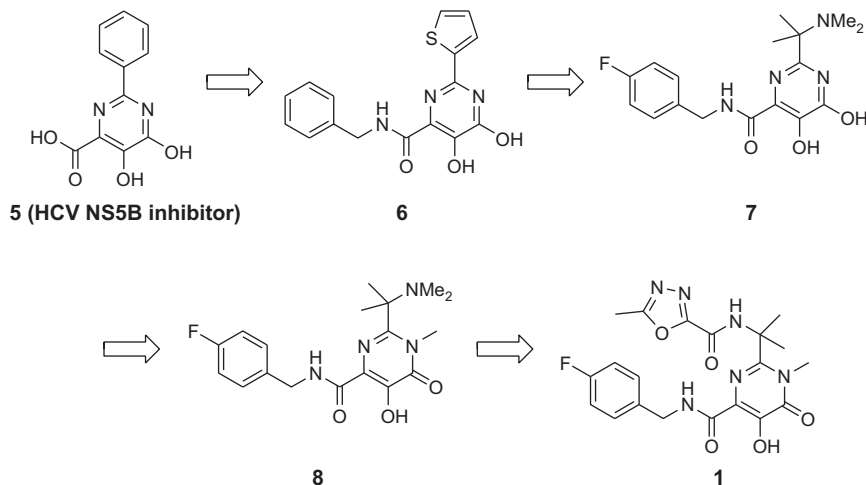


Figure 6.3 Lead progression for the discovery of raltegravir.

potency (85 nM) in an integrase strand transfer assay in return for this very simple modification. Modification of the 2-position of the pyrimidine led to further improvements and substitution of the nitrogen to form the pyrimidinone in moving from **7** to **8** was the last major structural core change of note. Finally, continued optimization around the aminomethyl C2 substituent allowed for the right balance of physicochemical properties while maintaining cell penetration and limiting protein binding effects to deliver exceptional potency. The result was raltegravir (**1**).³⁹

Since the approval of RAL, the inclusion of an HIV-1 integrase inhibitor as part of highly active antiretroviral therapy (HAART) regimens has become increasingly attractive.^{40,41} Integrase inhibitors have been well tolerated and among the most potent antiretrovirals discovered to date, based on nearly 5 years of post-launch experience with raltegravir, in addition to numerous clinical studies with experimental agents. In addition to exceptional viral load reduction, the class appears to result in a more rapid decrease in RNA copies, although it is still unclear if this results in a meaningful clinical benefit.⁴²

Raltegravir had the notable privilege of being a first-in-class agent after several years of relative calm in the development of new antiretroviral drugs. The field was thus primed for an agent with a novel mechanism and the stage was set for a pair of identical pivotal Phase 3 trials, based on geographic regions and with a placebo control in a treatment-experienced population^{43,44} that would demonstrate the superiority of raltegravir when added on to optimized background therapy (OBT) with a placebo control in a treatment-experienced population. The combined BENCHMRK 1 and 2 results at 48 and 96 weeks showed clear superiority of a 400 mg twice-daily (bid) dose of raltegravir over OBT alone. In addition to the primary endpoint of the number of patients being below either 400 or 50 copies HIV RNA per mL, a significant increase in CD4⁺ cell numbers per mm³ was noted for the raltegravir arms over the

placebo. Furthermore, an analysis of resistance provided the first clear information on what has become known as RAL signature mutations: N155, Q148 and Y143, usually in combination with at least one additional mutation, especially in the case of the Q148 pathway, which favors a second G140 mutation to rescue viral fitness.^{45–49}

The treatment-naïve patient STARTMRK Phase 3 trial was of non-inferiority design and compared RAL with the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz, both as part of a three-drug regimen that included the two nucleoside RT inhibitors (NRTIs) tenofovir and emtricitabine. This study showed non-inferiority of RAL, with the INI and NNRTI arms achieving 81 and 79% efficacy (<50 copies mL^{-1}) at 96 weeks.^{50,51} Similar levels of CD4^+ cell increase were also observed.

The above studies clearly establish RAL as a bid option for the treatment of naïve or experienced patients and investigators at Merck sought to evaluate further the potential of their new agent. The SWITCHMRK study was an attempt to show efficacious non-inferiority to switching from a protease inhibitor (PI) to the INI RAL.⁵² Since PIs have a long history of deleterious effects on lipid profiles, albeit in the face of tremendous efficacy and high genetic barrier to resistance, it would be desirable to have a similar virological outcome without the lipid changes. However, RAL was shown to be inferior to lopinavir/ritonavir at 24 weeks. *Post hoc* analysis showed that if previous failure was taken into account, RAL performed similarly to the PI; unfortunately, this was not considered in the trial designs. The effects on cholesterol and triglycerides, however, were significantly more favorable for the RAL arm. Thus, from an efficacy standpoint, there was still interest in further demonstration of the ability to change from a PI to an INI. A slightly redesigned and smaller study called SPIRAL was undertaken in Spain to revisit the switch challenge. Ultimately, this study did show non-inferior efficacy and improved lipids in a 48 week trial, when patients with sustained suppression for more than 6 months were chosen to switch regimens.⁵³

These switch studies, along with the levels of resistance observed, particularly in the BENCHMRK studies, began to create concerns about the genetic barrier to resistance for RAL and perhaps, more broadly, for the class in general.⁵⁴ The final major clinical study of note involving RAL was an attempt to gain approval for once-daily dosing.⁵⁵ The QDMRK study was designed to compare 400 mg bid with 800 mg qd, with a common backbone of tenofovir/emtricitabine. A fully powered Phase 3 study was planned through 96 weeks of dosing, with primary endpoints at both 48 and 96 weeks. The 48 week analysis showed a slightly inferior performance of the qd arm of the study when all participants were included, with an apparent worsening situation for patients with high viral loads of $>100,000$ copies mL^{-1} or low CD4^+ cell count.

It is interesting to compare the above results with early clinical and preclinical data around the PK profile of RAL. From the healthy volunteer studies, RAL showed a rapid initial (α) elimination phase ($t_{1/2} = 1$ h) and a longer terminal (β) elimination ($t_{1/2} = 7\text{--}12$ h).⁵⁶ Similar PK was observed in the Phase 2a study, which established antiviral efficacy after 10 days of monotherapy

when trough concentrations (C_{12h}) were maintained above a 33 nM IC_{95} .⁵⁷ Interestingly, all doses tested, 100–600 mg bid, showed similar viral load responses during this study, making a dose–response curve difficult to assess fully. Further review of the preclinical PK data showed a similar biphasic elimination curve, with a rapid initial phase followed by a slower terminal phase. Although specific data were not reported for RAL for the early-phase half-life in rat, dog and monkey studies, it was mentioned that RAL showed similar PK to a closely related compound that nicely mimicked the clinical data from the healthy volunteer and monotherapy trials. Overall, RAL showed a 2, 11 and 4 h terminal elimination phase in rat, dog and rhesus i.v. PK studies, respectively. The corresponding clearance values were 39, 6 and 18 mL min⁻¹ kg⁻¹; volume of distribution (V_d) measures were 2.0, 0.9 and 1.2 L kg⁻¹.

Elvitegravir (EVG) is derived from a quinolone antibiotic scaffold originally designed for bacterial DNA gyrase activity. The evolution of the quinolone into a potent INI is shown in Figure 6.4a. Shinkai and co-workers designed the quinolone keto acid motif **10** as a bioisostere of the two-metal binding compound L-870,810 (**9**), which was an early-generation INI and the first to show clinical efficacy.⁵⁸ Although the keto acid **10** did not have integrase activity, researchers were surprised to see that the structurally simpler acid **11** had low micromolar inhibition of an enzymatic strand transfer assay. Through significant lead optimization, workers at Japan Tobacco were able to build in substantial potency (IC_{50} = 0.9 nM, protein-adjusted IC_{50} = 9.8 nM) and develop a suitable PK profile to justify clinical development.⁵⁹ Preclinical PK showed moderate oral bioavailability in rats and dogs (34.1 and 29.6%) and also modest i.v. clearance rates (0.5 and 1.0 L h⁻¹ kg⁻¹), respectively. Terminal half-life measures were 2.3 and 5.2 h in rats and dogs, respectively.⁶⁰

EVG is predominantly metabolized via CYP3A. This feature became evident during the early clinical investigations and has manifested itself throughout the EVG development program.^{61,62} As a result, EVG requires a PK booster co-dose to achieve a clinically meaningful increase in EVG AUC (area under the curve) measures, which is the key to making EVG a once-daily drug.^{63,64} Since the very early monotherapy efficacy investigations, all clinical studies have included a co-dose of ritonavir,⁶⁵ which itself is an early-generation HIV protease inhibitor, or of the CYP3A inhibitor cobicistat (COBI), which is based on ritonavir but devoid itself of potent HIV antiviral activity.⁶⁶ With the pharmacokinetic booster available along with the two nucleoside analogs tenofovir (TDF) and emtricitabine (FTC), the focal point of the development strategy for elvitegravir has been the four-drug combination pill (QUAD) of EVG–COBI–TDF–FTC.^{67,68}

Two key Phase 2 studies looked at EVG in either experienced or treatment-naïve patients. In the experienced population, a protease inhibitor was compared with EVG both with study arms including ritonavir and OBT.⁶⁹ For naïves, the QUAD regimen was compared with the three-drug combination containing Atripla (efavirenz–tenofovir–emtricitabine).⁷⁰ In both cases, the primary endpoints were robustly met and EVG progressed into pivotal Phase 3 studies. The first Phase 3 trial to report was a study in experienced patients

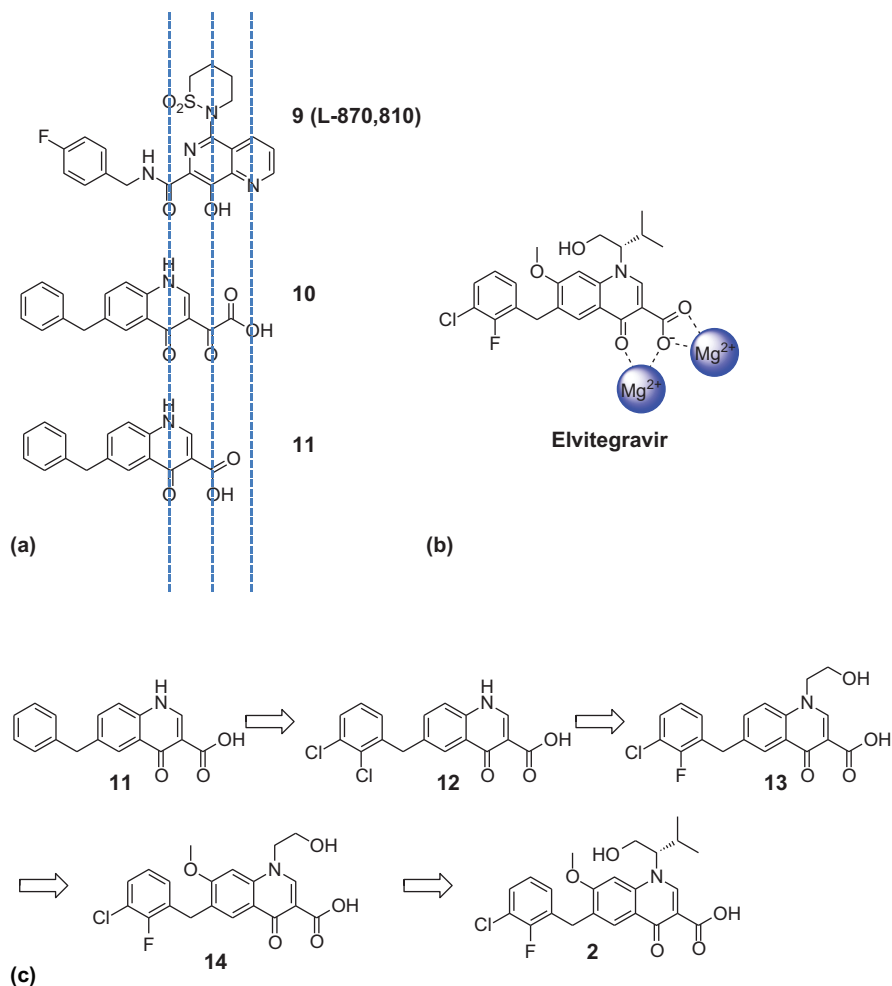


Figure 6.4 (a) Metal-chelating Lewis basic groups in the quinolone series derived from naphthyridone INI L-870,810. (b) Two-metal chelation model for elvitegravir. (c) Quinolone series evolution to discover elvitegravir.

with at least two-class resistance comparing EVG (150 mg qd) with RAL (400 mg bid) with a background regimen of a ritonavir-boosted protease inhibitor. EVG was shown to be non-inferior to RAL in the 48 week analysis.⁷¹ A second study (Study 102) based on the Phase 2 naive design mentioned above comparing two single-pill regimens has recently reached the 48 week analysis. The results confirm non-inferior efficacy of the QUAD to Atripla, with some advantages reported for CNS, rash and lipid levels favoring the integrase-containing regimen.^{72,73} A third study (Study 103), again in naive patients, reported non-inferior results when the QUAD regimen was compared with the boosted protease inhibitor atazanavir with a Truvada (tenofovir–emtricitabine) backbone, effectively comparing the boosted INI with a boosted PI.^{74,75}

With the advent of the QUAD regimen, elvitegravir clearly marks an advance in convenience and, as a result, likely also an improvement in adherence compared with raltegravir. However, EVG is highly cross-resistant to two of the three major RAL signature mutation pathways. The N155H and various residue 148 and 140 combinations were selected for in-cell culture passage experiments^{76–79} and have been observed in patients.^{80,81} EVG signature mutation pathways also include T66A/I/K and E92Q/V.⁷⁷ EVG does retain potency against the Y143 mutants,^{82,83} a difference from RAL that is well explained from seminal structural work done on the prototype foamy virus (PFV) surrogate system for HIV integrase by Cherepanov and co-workers.^{26,27} Tyrosine 212 in the PFV system corresponds to residue 143 in the HIV integrase protein. Raltegravir makes a π -stacking interaction between its oxadiazole tail with the wild-type tyrosine benzyl ring. EVG lacks an aromatic tail in the same region, hence its potency does not depend on this additional interaction with the protein.

The work mentioned above covering RAL and EVG is just a summary of the early-through late-stage development for the two integrase inhibitors. When looking over the data profiles of these drugs, the opportunities for improvement become apparent, centered around dosing requirements and resistance. These limitations were apparent very early during the development of both compounds and it was our goal to address both of these areas in our attempt to find a truly next-generation INI.

6.3 Discovery and Development of Dolutegravir

6.3.1 Differentiation Objectives

The current financial hurdles to bringing a drug from early discovery to market are enormous: some estimate the cost at over \$1 billion.⁸⁴ Although the actual number is hotly debated, the cost is high enough that the decision to develop a promising lead has significant ramifications and investment must be placed where it will have the greatest impact. With RAL and EVG already well in place, the development of an additional integrase strand transfer inhibitor needs to provide something of value to the patient, rather than being just a ‘me-too’ drug of questionable utility.⁸⁵ As our team began to consider the attributes necessary to deliver an impactful drug, we developed four critical primary criteria. These key attributes were (1) unboosted once-daily dosing, (2) a low-milligram dose burden that would be amenable to a variety of fixed-dose combinations (FDCs), (3) a superior resistance profile to retain potency against clinically relevant INI resistance mutations and (4) a high genetic barrier to resistance.

6.3.2 Design of a Next-generation Scaffold

Since the DKA motif was identified, many two-metal binding scaffolds other than a hydroxypyrimidinone (RAL’s core unit) or a quinolonecarboxylic acid (EVG’s core unit) have been explored in the field of HIV-1 integrase drug discovery. This

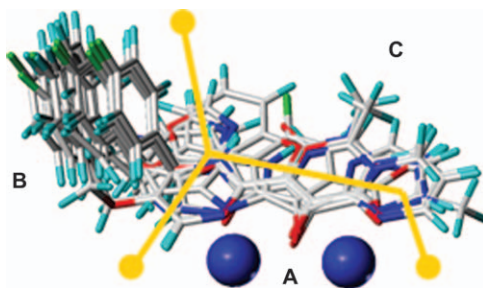


Figure 6.5 Overlay of accumulated two-metal binding scaffolds for refinement of pharmacophore elements. Yellow lines partition the structures into each pharmacophore region, A, B and C. Reproduced with permission from reference 90. Copyright (2012) American Chemical Society.

long-term accumulation of SAR experience, based on the two-metal pharmacophore concept, opened up a new vista of lead discovery, which would eventually address all the key attributes mentioned above. Figure 6.5 represents a structural overlapping model of many historical two-metal binding scaffolds. As elaborated in the original pharmacophore discussion above, region A is the two-metal binding motif critical to all members of this class of strand-transfer active-site binders. Region B is a hydrophobic pharmacophore space which is consistently a halo-substituted phenyl group for optimal potency based on an empirical review of many molecules in this class from our laboratories and others. Region C is commonly fairly flexible and tolerant to diverse structural modifications that allow for optimization of the PK and other drug-like properties required of an orally dosed small molecule. Additionally, there has been evidence of region C adding significantly to the intrinsic potency of the inhibitor.

Region B was an interesting area in which we began to focus on subtle details during our molecular design of a novel scaffold. Phenyl rings are clearly clustering on region B, but upon closer inspection there appeared to be a range of space occupied by the different rings, which have antiviral potencies ranging from nanomolar to micromolar. It appeared from many examples that a molecule extending the phenyl ring further in the overlap comparison was more likely to show higher potency. Figure 6.6 shows two examples of a comparison with different spacing of the region B hydrophobic benzyl group.

In order to demonstrate the concept, we can use a series of naphthyridines (NAP-A and -B) and naphthyridinones (NTD-A and -B) as examples. Both NAP-A and NAP-B have an identical naphthyridine metal chelation motif, but are effectively rotated on a pseudo C_2 symmetry axis, placing the benzyl group on opposite ends of the heterocycle.^{86,87} As such, the NAP-A and NAP-B scaffolds are set up to bind in a reversed orientation to one another, thereby placing the hydrophobic benzyl group in the same pharmacophore space. As seen in an overlapped model in the middle, NAP-A spaces the phenyl ring further from the core region and represents higher potency than NAP-B. The situation is similar for the naphthyridinones NTD-A and -B.^{88,89} Again, the

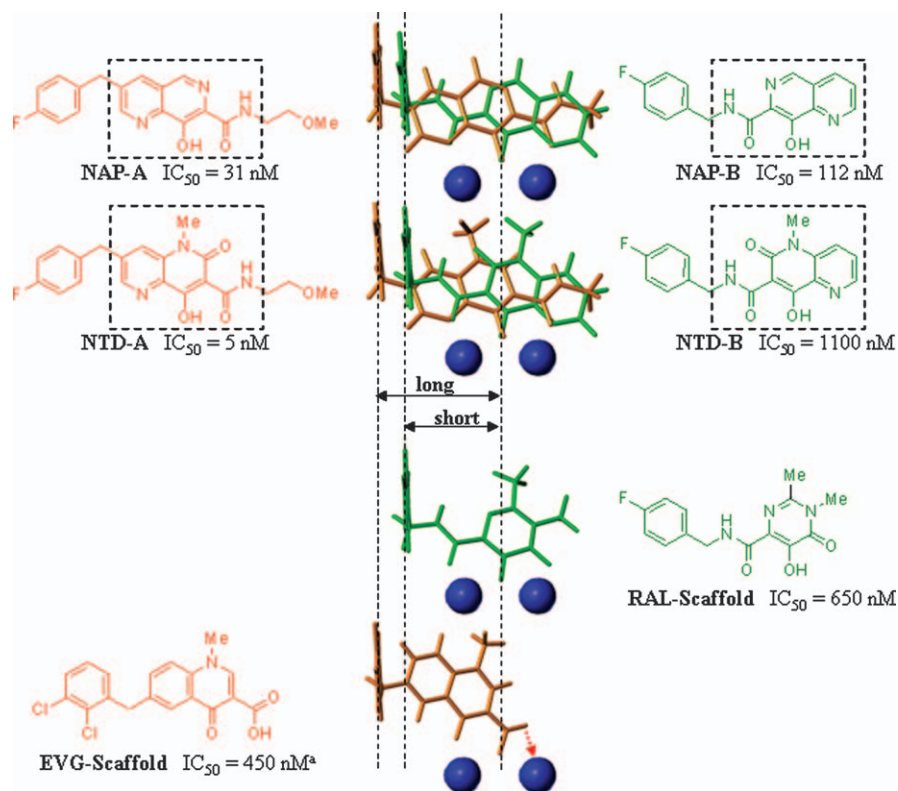


Figure 6.6 Comparison of hydrophobic region B aromatic ring spacing in pharmacophore analysis and design. The basis for increased ring spacing for optimal potency in the design of a next generation inhibitor.

^aData from reference 59. Reproduced with permission from reference 90. Copyright (2012) American Chemical Society.

NTD-A further extends the hydrophobic moiety and shows higher potency than NTD-B.

Additional observations with other scaffolds further supported this optimal hydrophobic positioning and, as a result, we adopted this rationale in the course of our molecular design. Interestingly, a simplified RAL scaffold, which has a short benzyl group spacing, has only weak to modest antiviral potency. However, as we saw in the RAL optimization story (referenced in Section 6.2), with additional modifications towards region C, RAL also achieved low nanomolar potency.

In contrast, the EVG scaffold extends a phenyl group similarly to the NAP-A and NTD-A structures. However, metal chelation could not be completed as effectively due to weak bidentate coordination with the carboxylic acid unit. Again, with additional modifications to the quinolone carboxylic acid scaffold, EVG achieved high potency. In recent crystallographic studies of the prototype form virus (PFV) intasome with some HIV-1 integrase inhibitors reported by

Cherepanov and co-workers, these aromatic rings extended to region B appear to contact with the viral DNA substrate cytosine base.²⁵ This further supports the hypothesis that RAL could not extend a phenyl group sufficiently deep to stack effectively with the substrate base. These crystallographic views also support the suggestion that the EVG carboxylic acid could not complete an effective bidentate chelation to both divalent magnesium cofactors in the active site. Although these inspections are based on complexes with the PFV intasome, the HIV-1 intasome likely represents a similar scenario.

The next step for molecular design was to consider the metal chelation properties in region A, which is the critical pharmacophore. The DKA original scaffold in this class includes a three-oxygen chelating unit. However, medicinal chemistry optimization led to new two-metal binding scaffolds, including basic heteroaromatic nitrogen atoms involved in metal coordination. The idea is that a more basic chelator is able to provide higher potency, in addition to designing chelating motifs into more ‘drug-like’ structures, as well as providing easier synthesis. Several of the early candidates mentioned above (S-1360, L-870810, GSK364735, GS-9160, BMS-707035) include at least one nitrogen to coordinate a metal cofactor. The early-generation NTD and NAP scaffolds described previously also consist of a nitrogen–oxygen–oxygen chelating unit. As is generally accepted, integrase uses divalent metal cofactors under physiological conditions that are presumed to be Mg^{2+} .^{21,22} According to the hard and soft acid and base (HSAB) theory, ionic magnesium is categorized as a hard metal; therefore, a hard base, such as oxygen, is preferred over nitrogen to coordinate a magnesium metal in the active site. hence we proposed replacing the nitrogen in NTD-A with oxygen in the chelation unit. An additional benefit of using a harder base, such as oxygen, is to reduce potential side effects caused by non-specific binding to soft metals utilized in other biological functions in the human body. Our first attempt at replacing the nitrogen with an oxygen is shown in Figure 6.7.⁹⁰ Owing to bonding differences in oxygen versus nitrogen, the ring system in the NAP and NTD scaffolds was opened to utilize an ‘acyclic’ carbonyl as the Lewis basic chelation group. The prior scaffold ‘ring’ integrity and positioning of the benzyl group were maintained by placing a carboxamide in the 5-position of the pyridone system whereby an intramolecular hydrogen bonding with the pyridone core carbonyl allowed for coplanarity and

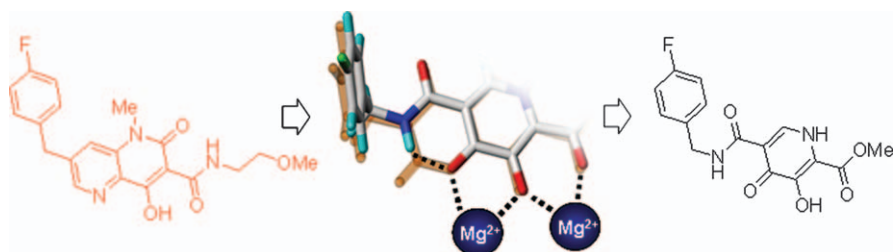


Figure 6.7 Molecular design of N to O chelation motif changes and introduction of the carbamoylpyridone metal chelation region A.

re-establishment of the original scaffold pseudo-ring system. With this design, the benzyl is placed in the desired hydrophobic pharmacophore region, thereby maintaining a favorable region B alignment.

An initial carbamoylpyridone inhibitor **15** provided a high antiviral potency against a wild-type HIV-1 strain from a very simple scaffold. Even in the presence of human serum albumin (HSA), a potency reduction of only 12-fold resulted in 120 nM (38.4 ng mL^{-1}) as the protein adjusted IC_{50} in an MT4 cell-based antiviral assay ($^{\text{MT4}}\text{PAIC}_{50}$). This agent did not show sufficient efficacy against three clinically relevant RAL-resistant mutants (Table 6.1); nevertheless, it was comparable or slightly improved when compared with RAL or EVG. Furthermore, animal PK studies provided good $t_{1/2}$ (3.79–5.31 h), consistently low i.v. clearance ($\text{Cl}_t = 0.84\text{--}2.44 \text{ mL min}^{-1} \text{ kg}^{-1}$) and moderate bioavailability ($F = 22.2\text{--}53.4\%$) across species, with the rat data shown in Table 6.2. Most importantly, good coverage of plasma concentration over the $^{\text{MT4}}\text{PAIC}_{50}$ of 38.4 ng mL^{-1} was observed in every species at 24 h post-dosing (5 mg kg^{-1} oral dose). Moderate molecular weight and good solubility combined with neither significant CYP inhibition nor metabolic concern suggested that this was an excellent starting point for further optimization.

A first attempt at further modification was the replacement of the C2 ester unit with an amide unit. It was unsuccessful because of an unfavorable intramolecular hydrogen bonding or steric hindrance that breaks the planarity of the chelating core unit, which is required for effective binding to the two active site metals (data not shown). Cyclization of the amide unit was a logical means to address both removal of the ester and alleviation of the amide planarity issue in order to reconstruct the metal chelation motif coplanarity. Hence this bicyclic carbamoylpyridone core unit was derivatized towards cyclized subclasses, represented by **16–18** shown in Tables 6.1 and 6.2. The inhibitors are differentiated by a linkage, by the presence of unsaturation in the new ring or by the addition of a hydroxyl group, in the case of **18**. As was expected, every derivative exhibited high antiviral potency against the wild-type strain and acceptable potency shift in the presence of HSA. Although inhibitors **16** and **17** do not have sufficient efficacy against Q148K, one of resistant mutants for RAL, inhibitor **18** showed improvement with only a 2.1-fold change. Conversely, the rat PK profiles for bicyclic analogs **16** and **17** were encouraging, but low oral bioavailability of the hydroxyl derivative **18** raised concerns and left room for improvement. In addition, the hemiaminal functionality is not only a chiral center but quickly racemized after chiral separation, presumably via equilibrium of the open-chain aldehyde form.

As had been done in progressing from the monocyclic amide to the bicyclic derivatives to address the issue around coplanarity and its effects on potency, we again resorted to the formation of a ring to solve a completely different challenge in the case of the hydroxyl analog **18**. Formation of the tricyclic ring system **19** again resulted in high potency against the wild-type virus and a low protein-adjusted IC_{50} and, similarly to the hydroxyl derivative, the tricyclic version had only a threefold change against the Q148K site-directed mutant that was being used as a surrogate measure of the ability to address clinically

Table 6.1 Antiviral activity for carbamoylpyridone integrase inhibitors.

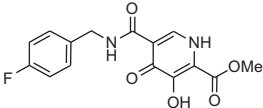
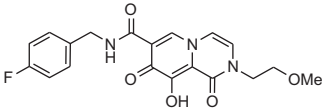
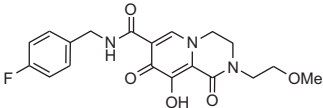
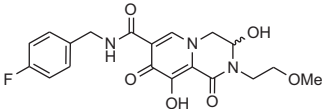
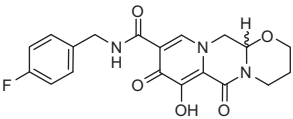
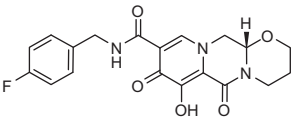
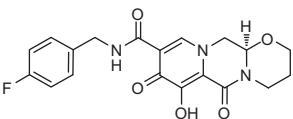
Compound	Structure	<i>MT4</i> IC ₅₀ (μM)	<i>MT4</i> PAIC ₅₀ (μM)	<i>Q148K</i> (FC)	<i>N155H</i> (FC)	<i>Y143R</i> (FC)
RAL		0.0062	0.029	83	8.4	16
EVG		0.0013	0.029	>1700	25	1.8
15		0.010	0.120	200	21	1.5
16		0.0016	0.027	35	3.7	1.6
17		0.0059	0.018	18	1.5	1.5
18		0.0038	0.014	2.1	1.5	1.5

Table 6.2 Rat pharmacokinetics for carbamoylpyridone integrase inhibitors.

Compound	Cl ($ml\ min^{-1}\ kg^{-1}$)	$t_{1/2}$ (h)	V_{dss} ($L\ kg^{-1}$)	F (%)	$C_{24}\ h ^{MT4}PAIC_{50}$
15	1.16	4.67	0.19	53.4	3.4
16	0.02	10.5	0.01	39.5	2600
17	0.51	3.36	0.12	40.6	27
18	0.24	7.6	0.14	13.6	117

Table 6.3 Antiviral activity and pharmacokinetics for **19**.

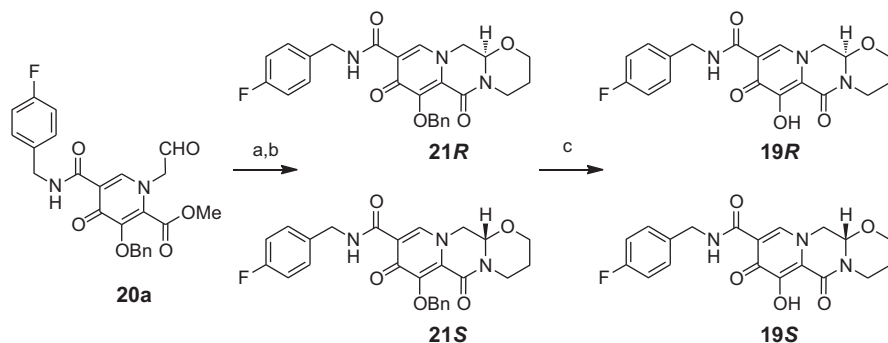
Compound	Structure	$pHIVIC_{50}$ (μM)	$pHIVPAIC_{50}$ (μM)	Q148K (FC)	Cl ($ml\ min^{-1}\ kg^{-1}$)	F (%)
19		0.002	0.019	2.8	0.10	51
19S		0.002	0.081	2.4	0.10	79
19R		0.002	0.009	11	0.17	52

relevant resistance mutations. Also encouraging was the fact that the tricyclic compound **19** had a very low i.v. clearance in rats, along with the best half-life observed thus far and over 50% oral bioavailability (Table 6.3).

6.3.3 Execution and Delivery of the Tricyclic Carbamoylpyridone

Once robust activity and scaffold properties had been established, our program moved to a single-round pseudotyped antiviral luciferase reporter assay (pHIV),⁹¹ for improved throughput and biosafety concerns. Tricyclic carbamoylpyridone **19** was also a potent antiviral (IC_{50} 2 nM) in the pHIV assay while demonstrating a similar threefold loss in activity against the Q148K mutant virus, as was observed previously in the MT4 assay system. Additionally, a modest protein binding shift of tenfold resulted in a protein-adjusted IC_{50} ($pHIVPAIC_{50}$) against the resistant virus of 53 nM.

This virological profile was a significant advance toward our goal. However, this data set was from the racemate and the concern over differential potency,



Scheme 6.1 Synthesis and isolation of tricyclic carbamoylpyridone enantiomers **19R** and **19S**. Reagents and conditions: (a) 3-aminopropanol, AcOH, μ W; (b) chiral SFC separation; (c) Pd/C, H₂, THF.

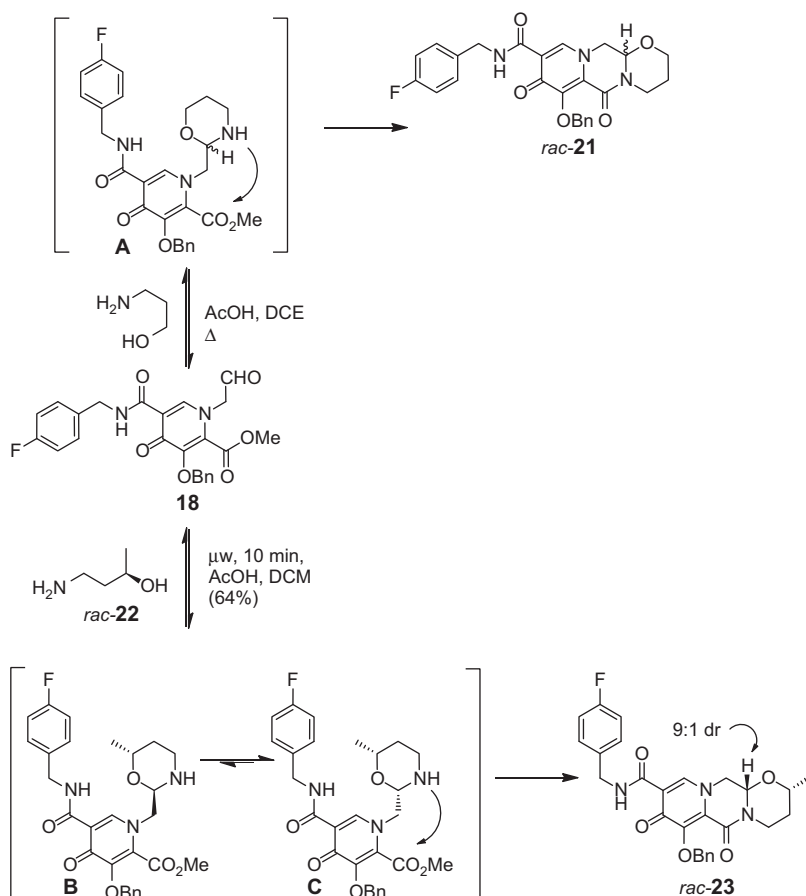
protein binding and PK properties needed to be addressed immediately. The individual isomers were isolated after chiral chromatographic separation of the *O*-benzyl-protected precursor **21** to give the enantiopure benzyl ethers **21R** and **21S**, which were subsequently deprotected through hydrogenolysis to give the individual pure enantiomers of **19** (Scheme 6.1).

Table 6.3 gives comparison antiviral and key low-dose rat PK parameters for racemic **19** and its individual enantiomers **19S** and **19R**. We were initially concerned that the hemiaminal stability would be an issue, but no inter-conversion or chemical stability issues were observed with the purified isomers of **19** or any other of the tricyclic analogs discussed below. Notably, the isomers had almost identical antiviral activity, although significantly different protein binding shifts, with the *S* isomer showing a 40-fold shift, whereas the *R* isomer had a very modest 5-fold loss of potency with added human serum albumin. The isomers also showed a differential loss of activity against the Q148K mutation, with the *S* derivative having only a 2-fold potency change, whereas the *R* analog had a 11-fold shift in activity. Both purified isomers and the parent racemate showed very low rat i.v. clearance values and good oral bioavailability, further stimulating our interest in the series. However, close inspection of the data set makes it evident that data for the racemate are in fact a convolution of the favorable potency and low protein adjustment from the *R* isomer, while the PK and Q148K fold change are perhaps driven substantially by the *S* isomer.

Although the data may be complicated, the clear fact was that both enantiomers were very promising compounds. The question was how to progress, as separation via chromatographic means was highly undesirable, even for early stages of development. The most attractive way forward would have been an asymmetric synthetic approach but, since the stereocenter of concern was a hemiaminal in a complex ring system, good synthetic methodology was not apparent and would have required extensive experimentation even if it were possible under any conditions. The concept of a chiral auxiliary was among the first to be considered, but this was quickly recognized as an

undesirable approach because there was no precedence for our specific system and the fact that our core ring system had limited functional handles on which to append any necessary stereocontrol elements. Consideration of the mechanism for the condensation and ring formation brought to mind the use of substrate control as a possible strategy to establish a handle to impact stereochemistry and also further evaluate SARs. Our hope was that by introducing substitution to the system we would either further improve the biological activity and/or PK and allow for robust stereocontrol or minimally reap the synthetic chemistry benefits and not have any deleterious effects on the above properties. This concept is akin to an auxiliary approach whereby a diastereomeric situation is established to discriminate between isomers, with the exception that, in our case, the auxiliary is retained as a beneficial part of the final drug.

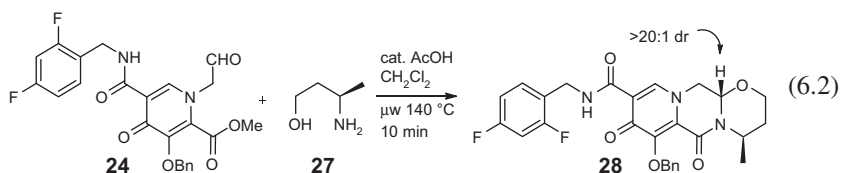
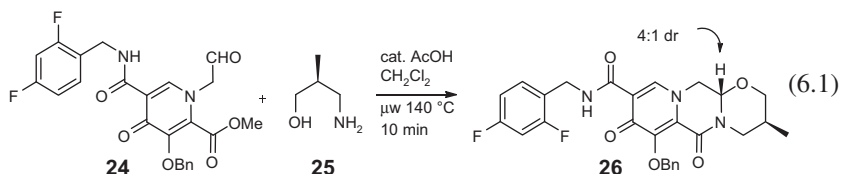
As shown at the top of Scheme 6.2, with the use of an achiral amino alcohol, as had been done for the synthesis of **21**, there is no stereochemical preference



Scheme 6.2 Implementing substrate control of a hemiaminal stereocenter.

in intermediate **A** and, therefore, racemic product is formed. However, if an amino alcohol with a chiral center is employed, diastereomeric intermediates **B** and **C** are formed, resulting in an equilibrium that should favor the most stable chair conformation, as indicated by intermediate **C**. The final step of lactam ring closure should then lead to a biased product distribution related to the intermediates **B** and **C**. In the first attempt at this method using a racemic alcohol, *rac*-**22**, a 9 : 1 diastereomeric ratio (*dr*) favoring the expected product **23** from having both the methyl and methylene hemiaminal substituent in equatorial orientations was observed. Since our initial experiment was only to prove the relative stereocontrol principle in our system, we immediately repeated the reaction with chiral non-racemic alcohol **22** and obtained enantiomerically pure **23** after removal of the minor diastereomer by achiral silica gel chromatography. Removal of the benzyl ether under standard hydrogenolysis conditions served as a final step to arrive at the desired inhibitor design.

We sought to extend this methodology further to other substituted 1,3-amino alcohols. In the case of the 2-substituted derivative, an erosion of the stereocontrol was observed under the conditions employed to give a still useful 4:1 selectivity [eqn (6.1)]. Placement of the stereocenter next to the amino terminus of the 1,3-amino alcohol led to exceptional diastereoselectivity [eqn (6.2)].



Interestingly, regarding the 3-substituted case shown in eqn (6.2), the stereochemical outcome resulted in the methyl and hydrogen of the two stereocenters existing on the same face, which effectively places the methyl group in an axial position. This may not be expected upon casual inspection of the system since, mechanistically, the reaction is believed to proceed through first formation of the hemiaminal with the aldehyde, which then can cyclize to form the tricyclic-fused amido system. This initial hemiaminal would be expected to exist in the most stable chair form indicated under energy local minima **B** in Figure 6.8. Ring closure of this would then provide the 1,3-diaxial hydrogen-containing system shown as **D** in Figure 6.8, but that is not observed.

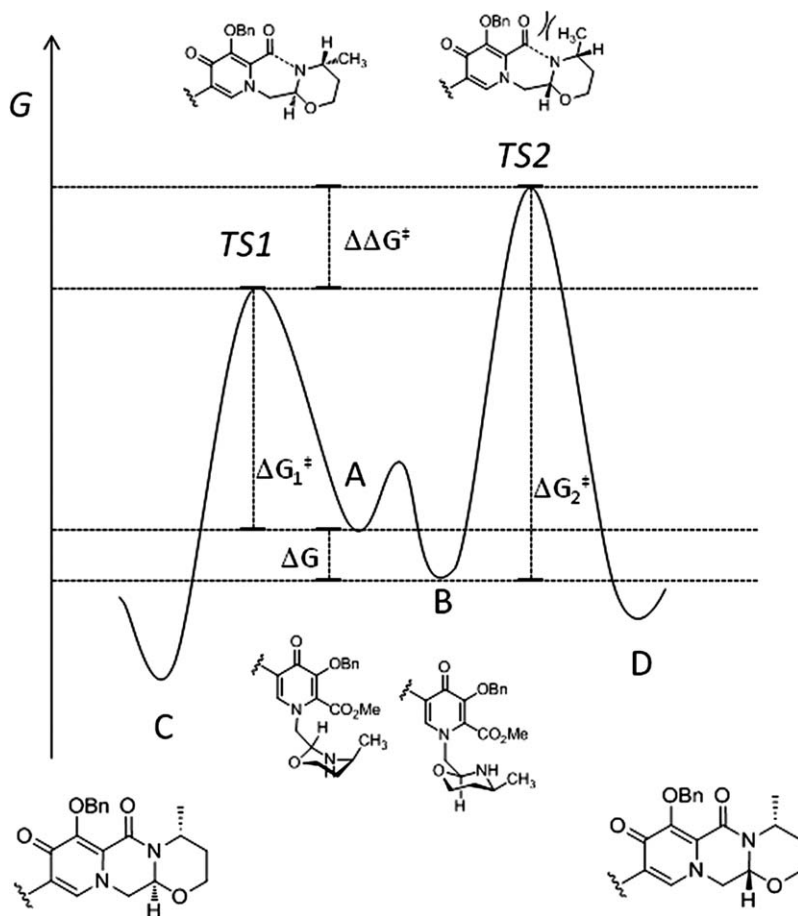
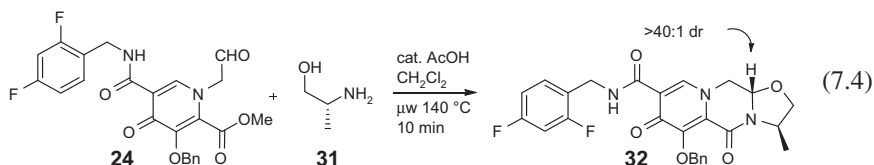
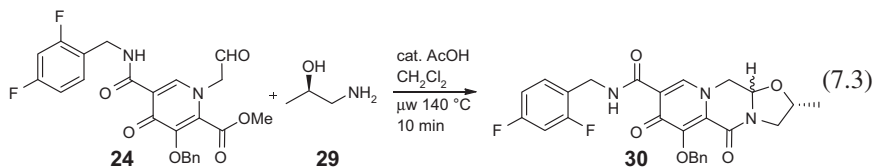


Figure 6.8 Curtin–Hammett explanation of 4-methyl substitution stereochemistry.

In fact, a Curtin–Hammett scenario is established as a result of a significant eclipsing interaction, resulting in a high-energy TS2 compared with the alternative axial methyl-containing TS1. There is a $3.6 \text{ kcal mol}^{-1}$ energy difference between the two diastereomeric products favoring C, which is observed experimentally as the predominantly observed product with $>20:1$ selectivity.

The findings for the 1,3-amino alcohols stimulated our interest to investigate the related 1,2-aminopropanols to explore the diastereoselectivity of this system. Our initial attempt using (*R*)-1-aminopropan-2-ol (**29**) gave disappointing results, with a difficult-to-separate 3:1 mixture of diastereomers (**30**) [eqn (6.3)]. However, switching to the regioisomeric (*R*)-2-aminopropan-1-ol (**31**) gave a $>40:1$ mixture of diastereomers favoring the *trans* stereochemistry as shown in **32** [eqn (6.4)]. This is again explained as a result of a Curtin–Hammett situation, whereby the energetically favored intermediate is

slow to cyclize and the product distribution is driven by the ring-closing step energetics, with the *trans* product having a ~ 2.24 kcal mol⁻¹ lower energy.



6.3.4 Choosing the Optimal Candidate

We were pleased to observe that the methyl isomers (**33–37**, **3**) all had high potency as good as or better than that of the unsubstituted original enantiomerically pure analogs of **19**. A detailed examination of the SAR is not included herein, but suffice it to say that the methyl analogs appeared to be the optimal molecules to deliver stereocontrol while preserving or improving the necessary virological and PK properties (Table 6.4). Further complexity, lipophilicity, polarity or size of the substituent did not appear to bring any advantages to justify the added challenges of accessing the necessary amino alcohol and increase in molecular weight.

A key parameter in our triage of compounds was the coverage of the protein-adjusted IC₅₀ at 24 h post-dosing from a standard 5 mg kg⁻¹ oral dose (C_{24h}/p^{PHIV}PAIC₅₀). The formulation studies ultimately do become important, but for screening of analogs we typically began with either a solution or suspension dose and eventually moved to a fully crystalline drug in capsules as a measure of our worst-case bioavailability. The combination of the PK profile along with early virology data places compounds **3** and **4** in an area with great potential for more detailed virological assessment.⁹²

6.3.5 Tricyclic Carbamoylpyridones Deliver ‘Next-generation’ Virological Profiles

Compounds **3** and **4** were examined in MT-4 cell and peripheral blood mononuclear cell (PBMC) HIV multi-round replication assays (Table 6.5).^{93,94} A more robust protein shift value was also determined *via* titrating various concentrations of human serum into the MT4 cell assay system.³⁵ From these data, a better approximation of the protein-adjusted clinical target was able to be determined. The overall fold shift values were somewhat higher than the

Table 6.4 Antiviral and pharmacokinetic data for diastereomeric carbamoyl-pyridone tricyclic series.

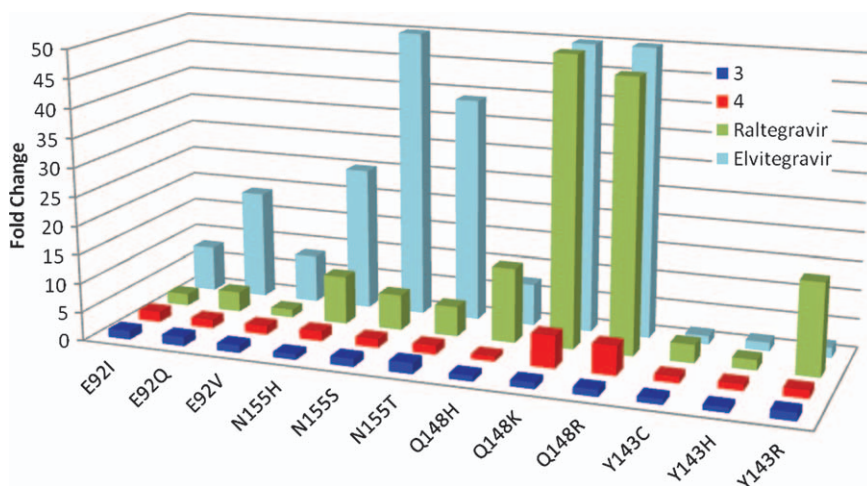
Compound	X	Structure	$p^{HIV}IC_{50}$ (μM)	$p^{HIV}PAIC_{50}$ (μM)	Cl (ml $min^{-1} kg^{-1}$)	C_{24h}^{pHIV} $PAIC_{50}$
33	H		0.002	0.025	1.4	21
34	H		0.001	0.003	16.5	3.6
35	H		0.0008	0.21	ND	ND
36	H		0.0007	0.056	ND	ND
37	F		0.0005	0.004	0.04	702
3	F		0.002	0.022	0.23	112
4	F		0.0005	0.030	NR ^a	1247
38	F		0.005	0.039	0.20	725

^a Not reportable due to prolonged exposure and extrapolation in excess of 20% of the total AUC.

pHIV data using purified human serum albumin suggested, but the overall comparison was similar, with **4** showing a significantly higher fold shift than **3**. The final clinical target was determined using the PBMC data and applying a fourfold factor to correct from the measured IC_{50} to approximated IC_{90} and

Table 6.5 Antiviral potency and protein-adjusted IC₉₀ of **3** and **4**.

Compound	INST IC ₅₀ (μ M)	MT4 IC ₅₀ (μ M)	PBMC IC ₅₀ (μ M)	HuS fold shift	PAIC ₉₀ (ng mL ⁻¹)
3	0.003	0.002	0.0005	75	64
4	0.003	0.001	0.0002	408	166

**Figure 6.9** Comparison of fold change in HeLa cells IC₅₀ of **3** and **4** against key IN resistance mutations.

applying the fold shift factor to that number. For **3** and **4**, the final PAIC₉₀ values were determined as 64 and 166 ng mL⁻¹, respectively.

Further evaluation of the resistance profile of **3** and **4** was undertaken in a HeLa cell assay with several site-directed mutant viruses meant to profile the two lead structures against a panel of relevant raltegravir and elvitegravir signature mutations. As can be seen in Figure 6.9, the fold change in activity when challenged with resistant mutations is vastly improved for **3** and **4** compared with RAL and EVG. Further data for **3** have been reported previously in a wider panel of mutants.⁹³

6.3.6 Preclinical Pharmacokinetics

The PK profiles of the two leads were determined for Sprague–Dawley rats, beagle dogs and cynomolgus monkeys (Table 6.6). For the purpose of rigorous assessment, oral doses were performed using a standard 5 mg kg⁻¹ dose from fully crystalline drug in a capsule to assess solid dosage form performance. For the purpose of this study, **3** was studied as its corresponding sodium salt, whereas **4** was dosed as the neutral molecule. Values for the i.v. data for both compounds appear to correlate well with the free unbound fraction, resulting in higher clearance values relative to hepatic blood flow in species with a larger

Table 6.6 Pharmacokinetic parameters for rats, dogs and cynomolgus monkeys for **3** and **4**.

Compound	Species ^a	Unbound (%)	Cl (mL min ⁻¹ kg ⁻¹)	t _½ (h)	V _{dss} (L kg ⁻¹)	F (%)	C _{24 h} /PAIC ₉₀ ^c
3	Rat	0.1	0.2	6.2	0.1	35	18
	Dog	4.6	2.2	5.2	0.3	35	4.6
	Monkey	0.9	2.1	6.0	0.3	25	0.76
	Human	0.7	—	—	—	—	—
4	Rat	<0.1	NR ^b	>18	NR ^b	NR ^b	57
	Dog	0.7	0.3	5.7	0.1	8	0.9
	Monkey	0.3	0.3	4.0	0.1	6	1.3
	Human	0.4	—	—	—	—	—

^aLiver blood flow (lbf) = 55/31/44 mL min⁻¹ kg⁻¹ for rat/dog/monkey.

^bNot reportable due to prolonged exposure and extrapolation in excess of 20% of the total AUC.

^cThe PAIC₉₀ value used was the clinical target of 64 ng mL⁻¹ for **3** and 166 ng mL⁻¹ for **4**.

unbound drug fraction. This is most pronounced in dogs for **3**, where a 4.6% free fraction results in clearance being ~7% of liver blood flow (lbf) [the lbf values used (mL min⁻¹ kg⁻¹) were 55 (rat), 31 (dog) and 44(monkey), according to Davies and Morris⁹⁵] whereas for rats the clearance is extremely low (<0.5% lbf), corresponding to a much lower free drug fraction as determined by equilibrium dialysis measurements. The data for monkeys are between for the rat and dog at 5% of lbf (44 mL min⁻¹ kg⁻¹ for a monkey). Similar trends are present for **4**, although it has a lower free unbound fraction across all species that results in very low clearance. The oral bioavailability of **3** is good from a suspension dosing formulation. Lower values were measured for **4** that was dosed from an unoptimized solid ‘powder in a capsule’ formulation, although coverage of the PAIC₉₀ was still possible across species. Both compounds were highly permeable (MDCK, P_{app} = 585 nm s⁻¹ for **3** and 425 nm s⁻¹ for **4**). However, solubility in simulated gastrointestinal (GI) fluid was low (130 and 42 µg mL⁻¹ for **3** and **4**, respectively), likely resulting in dissolution-limited absorption from our solid-dose formulation.

No significant inhibition of CYP3A4 or time-dependent inhibition was observed for either **3** or **4**. The compounds were also extremely metabolically stable ($t_{½}$ >180 min) in rat, dog, monkey and human S9 fractions and also rat and human hepatocyte incubations ($t_{½}$ >220 min). The combination of all the above PK data resulted in low predicted human qd doses (<100 mg d⁻¹) that would achieve the targeted C_{trough} levels.

6.3.7 Choosing a Lead and Back-up

With two advancing leads, it became necessary to make some decisions about a front-runner and a back-up to prioritize resources and aggressively move the best asset forward. However, in our case, both compounds were attractive and there was nothing to suggest clearly that one should be sidelined, although it

was not a case of everything being equal. Different PK properties and C_{trough} efficacy targets led us to consider whether perhaps one of these compounds did have an advantage that we had not yet accounted for in preclinical studies. Consequently, both compounds continued to proceed. In the end, the lower clinical target of **3** made it the priority and it quickly gained speed over the remaining compound **4**. There were some concerns about the protein shift and higher C_{trough} target for **4**, but these properties have thus far proven to be significant attributes, albeit with far fewer data in hand than for **3**. A description of how **4** is being developed is described in the next section. Compound **3** ultimately became what is known as dolutegravir (DTG, S/GSK1349572) and compound **4** is known as S/GSK1265744 (S/GSK744).

6.3.8 Clinical Development of Dolutegravir

Early Phase 1 clinical data for DTG demonstrated a 15 h half-life while producing a 25-fold coverage of the PAIC₉₀ *versus* C_{trough} from a 50 mg once-daily dose, without the need for a boosting agent.⁹⁶ These data were translated into a study design whereby once-daily doses of 2, 10 and 50 mg were given for 10 days as monotherapy to HIV-infected patients who were treatment-naïve or off-therapy experienced during a Phase 2a proof-of-concept study. The results from these doses showed log₁₀ viral load decreases in HIV-1 RNA copies per milliliter of plasma of 1.51, 2.03 and 2.46, respectively (Figure 6.10).⁹⁷ DTG was well tolerated during these studies while delivering an excellent impact on

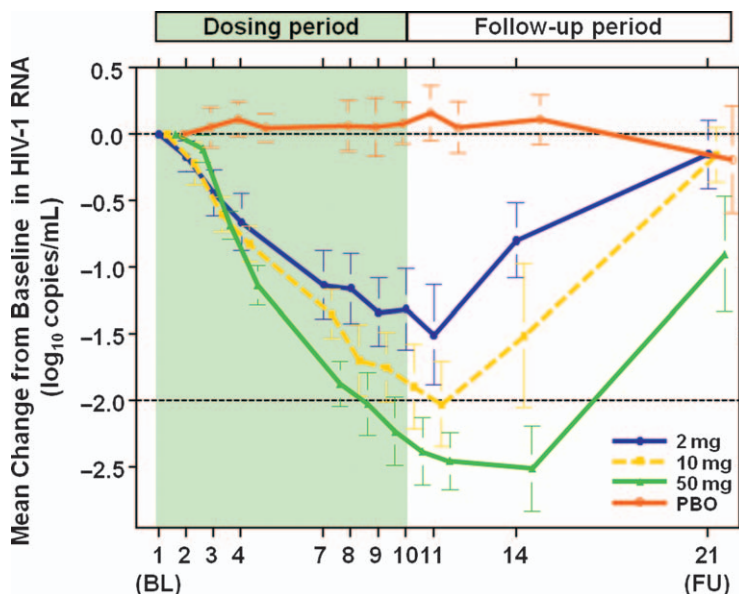


Figure 6.10 Phase 2a 10 day monotherapy viral load data for DTG.

viral load from a low once-daily dose. Clinical pharmacology studies showed no evidence of clinically significant drug–drug interactions with lopinavir/ritonavir (r), atazanavir/r, darunavir/r, tenofovir, the proton pump inhibitor omeprazole or multivitamins.^{98–101} Additionally, there appears to be a lack of a clinically significant food effect, further improving patient convenience and dosing flexibility.¹⁰² Antacids have been found to impact the clinical PK sufficiently that either dosing 2 h prior to taking antacids or 6 h after is recommended. This may be due to chelate formation in the stomach/GI tract with metal ions in antacids such as Maalox, which decreased absorption. It could also potentially be a result of pH adjustment in the stomach or upper GI compartment as a result of the basic supplement. It has been observed that co-administration with the NNRTI agent etravirine alone leads to significant decrease in exposure of DTG, likely due to induction of the UGT1A1 enzyme, a primary route of metabolic clearance for DTG.¹⁰³ If this combination is co-administered with a ritonavir-boosted protease inhibitor, this effect is greatly reduced.

DTG was further examined in the Phase 2b SPRING-1 dose-ranging study in treatment-naïve patients. Doses of 10, 25 or 50 mg qd with either Truvada or Epzicom nucleoside backbone agents were compared with the same background therapy with 600 mg of the NNRTI efavirenz (Figure 6.11).¹⁰⁴ Once-daily, unboosted DTG demonstrated durable antiviral activity for all dosing arms, with 88% responding through 96 weeks¹⁰⁵ and a favorable safety profile at the 50 mg qd dose, the dose chosen for further Phase 3 studies outlined below.

A second smaller but important dose-ranging study, termed VIKING, was designed to determine the antiviral effects of DTG in an integrase-resistant, highly treatment-experienced patient population. A first cohort of 27 patients

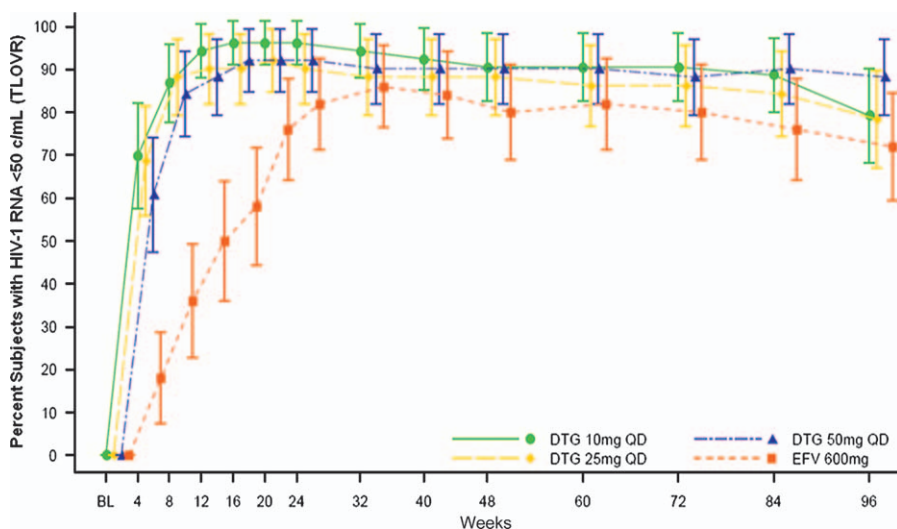


Figure 6.11 SPRING-1 Phase 2b treatment-naïve data through 96 weeks.¹⁰⁵

were given 50 mg qd on top of their existing background regimen for 10 days, followed by OBT on day 11 onwards. A second cohort of 24 patients were dosed under similar conditions, but with 50 mg bid to increase DTG drug levels further and a requirement for at least one fully active background agent. After 10 days with Cohort 1, 21/27 (78%) patients met the primary endpoint of <400 copies mL^{-1} whereas 23/24 (96%) of the Cohort 2 patients achieved the endpoint successfully. After 24 weeks, 41 and 52% of patients in the qd cohort and 75 and 83% of patients in bid cohort achieved plasma HIV RNA levels below either 50 or 400 copies mL^{-1} , respectively.^{106,107} These data are critical in addressing the ability of DTG to treat integrase-resistant viruses in an actual clinical environment and serves to validate the preclinical virological data presented above.

Phase 3 evaluation of DTG is well under way at the time of drafting this chapter. Two pivotal trials looking at the treatment-naïve population (SPRING-2 and SINGLE) have been reported thus far. SPRING-2 demonstrated that DTG was non-inferior to RAL through 48 weeks of treatment; 88% of study participants on the dolutegravir regimen were virologically suppressed (<50 copies mL^{-1}) *versus* 85% of participants on raltegravir [2.5% adjusted treatment difference, with 95% confidence interval (CI) -2.2% to $+7.1\%$].¹⁰⁸ SINGLE is an ongoing double-blind, double dummy study designed to compare the efficacy and safety of two antiretroviral regimens: dolutegravir 50 mg plus abacavir/lamivudine (Kivexa/Epzicom) *versus* Atripla (tenofovir/emtricitabine/efavirenz). The study demonstrated superiority of the dolutegravir-based regimen over Atripla: at 48 weeks, 88% of study participants on the dolutegravir regimen were virologically suppressed (<50 copies mL^{-1}) *versus* 81% of participants on Atripla [difference 7.4% and 95% CI $+2.5\%$ to $+12.3\%$; the difference in the primary endpoint was statistically significant, $p = 0.003$].¹⁰⁹

There are also two ongoing studies in the treatment-experienced population. The SAILING study compares DTG 50 mg qd OBT with RAL plus OBT in treatment-experienced patients who are failing in their current therapy. A fourth Phase 3 study, VIKING-3, will explore the effect of DTG treatment (plus OBT) in INI-resistant patients. At this point, DTG is in late-stage clinical evaluation, but sufficient data exist to consider the goals of the discovery program to have been robustly achieved.

6.3.9 Long-acting Parenteral INI – S/GSK744

The potency and resistance profile¹¹⁰ of S/GSK744 also makes it a very intriguing drug. A multipart Phase 1 study was conducted that included a cohort of HIV patients and also healthy volunteers to assess both efficacy and human pharmacokinetics, in addition to safety and tolerability data. In this study, subjects were given doses of 5, 10 and 25 mg once daily for 14 days and showed a near 30 h half life, with robust coverage of the PAIC_{90} value determined as 166 ng mL^{-1} . Even at the 5 mg dose, several multiples of exposure over the clinical target were observed through 72 h. In the monotherapy cohort,

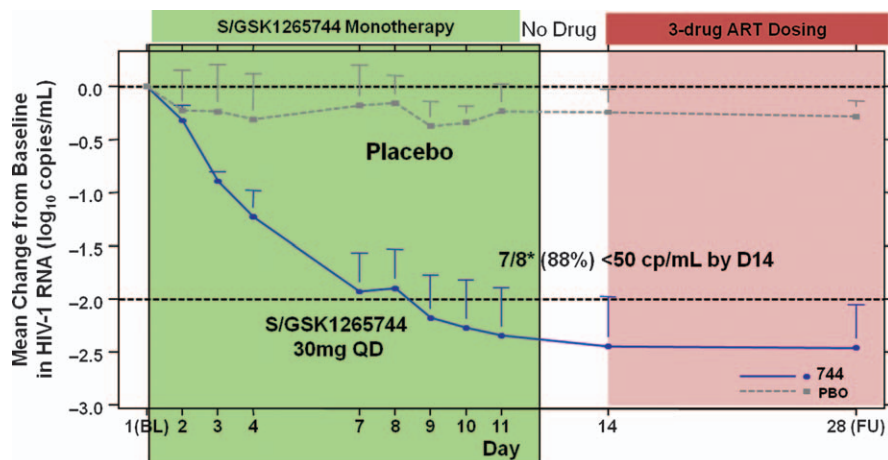


Figure 6.12 S/GSK1265744 Phase 2a monotherapy study.¹¹¹

a 30 mg qd dose provided a $2.6\log_{10}$ decrease in viral load at day 11, thus verifying the pharmacodynamic response predicted from the antiviral and PK data (Figure 6.12).¹¹¹ In addition, a study to examine drug–drug interactions between S/GSK744 and etravirine was completed and it was found that ETR does not affect S/GSK744 pharmacokinetics.¹¹²

The significant viral load impact observed along with the low dose required to achieve such an effect plus long $t_{1/2}$ led to the proposal for development of S/GSK744 as a long-acting parenteral agent. In this scenario, a suspension of drug is introduced *via* subcutaneous or intramuscular injection and a reservoir of drug is established whereby exposure is achieved through slow absorption from the drug depot. The first evidence of clinical PK, safety and tolerability of S/GSK744 in healthy volunteers has recently been disclosed, showing an apparent $t_{1/2}$ of 21–47 and 45–47 days from intramuscular and subcutaneous injections, respectively.¹¹³ Further studies are currently under way to examine this paradigm-shifting approach whereby daily adherence concerns are removed and new options for antiretroviral therapy are introduced in a situation that now requires relentless daily therapy for the remainder of a patient's life.

6.4 Non-catalytic Site Integrase Inhibitors

Despite the success of integrase strand transfer inhibitors, intensive research is ongoing to seek alternative mechanisms to inhibit this enzyme. The primary concern is the emergence of resistance in clinical use as observed with both raltegravir (RAL) and elvitegravir (EVG). To complicate the situation further, multiple studies have demonstrated cross-resistance between first-generation INIs, as alluded to above.^{49,77,79} Although the next-generation integrase inhibitor DTG has a superior resistance profile, the potential for resistance

remains, as is the case for all antiretroviral agents. Beyond strand transfer inhibitors, momentum has been growing in the field collectively known as non-catalytic site integrase inhibitors (NCINIs). These NCINIs are believed to be allosteric inhibitors of the integrase enzyme that disrupt the interaction of IN with the key cellular co-factor, lens epithelium-derived growth factor (LEDGF/p75). The proposed role for this interaction is to guide the preintegration complex to transcriptionally active regions of endogenous chromatin during integration. The LEDGF/p75 protein consists of 530 amino acids and contains a chromatin-binding N-terminal region that has a Pro-Trp-Trp-Pro (PWWP) motif, two copies of the AT-hook DNA binding motif and a nuclear localization signal (Figure 6.13). A highly conserved region of the C-terminus of LEDGF/p75 binds to IN and is termed the integrase-binding domain (IBD).^{114–118} The importance of LEDGF/p75 in HIV-1 replication has been demonstrated by numerous groups and it is well documented that alteration of the cellular co-factor by mutagenesis, knockdown, RNA interference or alteration of the nuclear localization signal sequence has a deleterious effect upon viral replication.^{119–122} Furthermore, LEDGF/p75 has been reported both to stimulate HIV-1 IN enzymatic activity (both strand transfer and 3' processing) and to protect it from ubiquitin–proteasome degradation.^{123,124}

In 2005, the first high-resolution crystal structure of the dimeric catalytic core domain (CCD) of HIV-1 IN complexed to the IBD of LEDGF/p75 was disclosed.¹²⁵ Of particular interest was a small binding pocket located at the CCD dimer interface where the IBD of LEDGF/p75 contacted two IN subunits (Figure 6.14). Although many of the key contact amino acid residues were validated by site-directed mutagenesis, additional interactions between the full-length proteins were more extensive than observed in the crystal structure.¹²⁰ A majority of these additional residues reside at the interface of the IN subunits and may suggest a potential role of LEDGF/p75 with IN multimerization.¹²⁶ Overall, these findings established the framework for designing small-molecule inhibitors of the interaction of LEDGF/p75 with HIV-1 integrase.

Debyser and co-workers utilized structure-based design to develop a series of inhibitors based on the aforementioned co-crystal structure of HIV-1 IN CCD with the IBD of LEDGF/p75.¹²⁷ Initially they performed a virtual screen

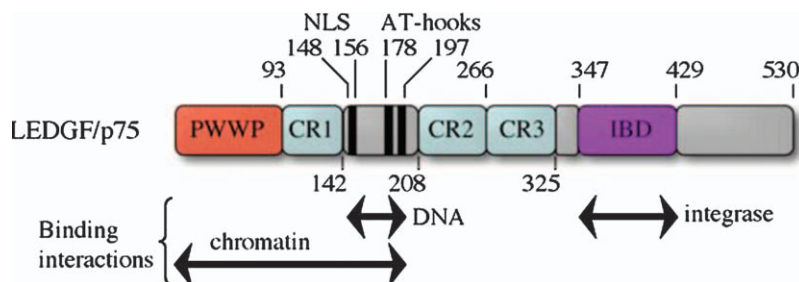


Figure 6.13 Domain organization of the LEDGF/p75 protein.

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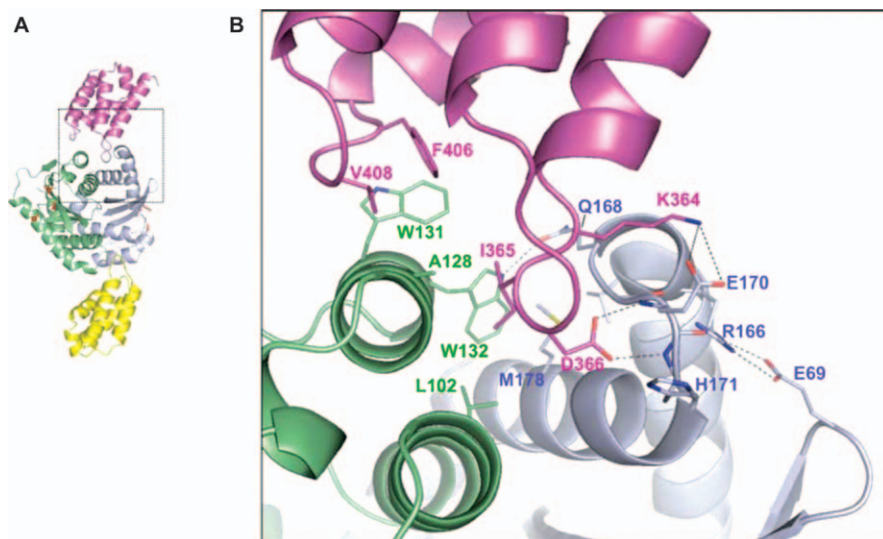


Figure 6.14 Crystal structure of the LEDGF/p75-IN Interaction. (A) Integrase-binding domain of LEDGF/p75 (in magenta and yellow) binding with the integrase CCD (in blue and green). (B) Highlighting key residues of the CCD-IBD interaction: a bidentate hydrogen bond of D366 with IN residues H171/E170 and I365 interacting with a hydrophobic pocket formed by IN residues A128, W132, L102 and M178. Reproduced under terms of the CCAL license from reference 118.

of 200 000 commercially available compounds that afforded 25 hits for further evaluation. This led to a series of 2-(quinolin-3-yl)acetic acid derivatives that inhibited the IN-LEDGF/p75 interaction *in vitro* and also demonstrated micromolar antiviral activity in HIV-1 infected cells with no appreciable cytotoxicity. Their most potent compound (LEDGIN 6, **39**, Figure 6.15) did not affect integrase–DNA binding, was a very weak strand-transfer inhibitor ($IC_{50} = 19 \mu M$) and was inactive in 3' processing ($IC_{50} > 250 \mu M$). *In vitro* resistance passaging of **39** selected for a single point mutation A128T, which is located at the edge of the LEDGF/p75 binding pocket (Figure 6.14B). The binding mode was unambiguously assigned, with a co-crystal structure of **39** revealing that the compound occupies the LEDGF/p75 pocket located at the CCD dimer interface. Furthermore, **39** demonstrated no significant cross-resistance with reverse transcriptase, entry or integrase-resistant virus strains. Conversely, an IBD-resistant strain of virus (A128T/E170G) was fully resistant to **39** but suffered no loss in susceptibility to the other antiviral agents tested. Although the lack of antiviral potency for these early inhibitors prevented further development, these compounds did offer valuable insight into the potential for this new class of integrase inhibitors.

Independent of the findings reported by Debyser's group, Boehringer Ingelheim published a patent claiming *tert*-butoxy(4-phenylquinolin-3-yl)acetic acid (tBPQA) derivatives as inhibitors of HIV replication.¹²⁸ This series was

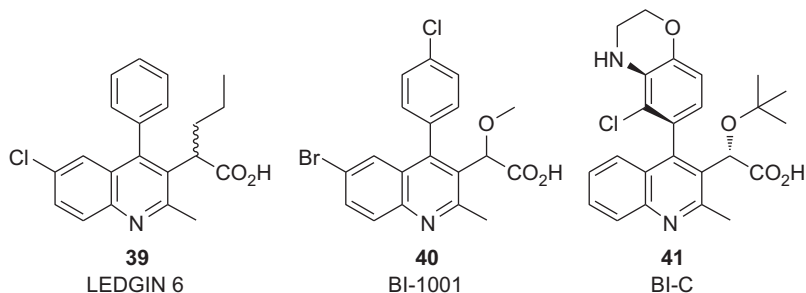


Figure 6.15 Structures of LEDGIN 6, BI-1001 and BI-C.

originally discovered as part of a high-throughput screen for integrase 3' processing activity (see Figure 6.15, **41** for a representative example).¹²⁹ The lead compound, BI224436, has excellent ADME and antiviral properties and was advanced into Phase 1a clinical trials to evaluate safety and dosing in healthy volunteers. Using the PhenoSense assay to measure antiviral activity against 200 clinical isolates, BI224436 had a mean IC_{50} of 13 nM. As observed with the LEDGIN class of inhibitor, there was no decrease in potency against RAL-resistant viruses.¹³⁰ In a double-blind, placebo-controlled, single-rising dose escalation, BI224436 proved safe and well tolerated up to the highest dose group of 200 mg. Overall, BI224436 demonstrated a dose-proportional increase in plasma C_{max} and AUC and was rapidly absorbed with a median t_{max} of 0.5 h and a $t_{1/2}$ of 7.1 h. Based on the PK profile, an oral solution dose of 100 mg qd provided the target therapeutic plasma drug concentration of 500 nmol L⁻¹ at 24 h.¹³¹ For undisclosed reasons, the development of BI224436 was stopped following the initial phase 1 study. In late 2011, Boehringer Ingelheim entered a licensing agreement with Gilead Sciences to develop this series of NCINI further.

Although the understanding of the mechanism of action for these types of inhibitors will likely evolve over time, it is clear that these molecules inhibit the HIV-1 IN enzyme in a manner unique to the current INIs. Owing to the structural similarity between the LEDGIN and tBPQA class of NCINI, it is difficult to reconcile the reported differences in mechanism of action between these molecules. A recent study refuted the original claim that LEDGIN 6 was devoid of 3' processing activity and reported it to be a low micromolar inhibitor of 3' processing (IC_{50} = 3.9 μ M) similar in activity to BI-1001 (IC_{50} = 2.3 μ M; **40**, Figure 6.15).¹³² Crystal structures for both **39** and **40** bound to the HIV-1 IN CCD reveal that the molecules occupy nearly identical spaces with the major difference being an additional H-bond interaction of the BI-1001 methoxy group with T174 of IN. Additionally, both classes of molecule promote integrase multimerization.¹³² This alternative mechanism has been well documented to play an important role in viral replication as precise coordination of the multimeric state of IN is required to bind viral DNA productively.^{126,133} The mechanistic details for inhibition are obviously complex, but a consensus appears to be forming around the concept of a

multimodal inhibition in which compounds impair both LEDGF-IN-dependent binding and LEDGF-independent integrase catalytic activity.

6.5 Conclusion

The emerging field of non-catalytic site integrase inhibitors is starting to gain significant momentum. The first molecules that inhibit the interaction of LEDGF/p75 with HIV-1 IN were reported in 2007, and in 2011 we already witnessed the first clinical trials of this new class of integrase inhibitor. The rapid progress in this new field is reminiscent of how the development of the strand transfer inhibitors accelerated over a decade ago. As with the active-site inhibitors, it would be expected that a number of molecules may be required to enter advanced preclinical and clinical study before medicinal chemists achieve success. It is exciting to consider potential regimens using both types of integrase inhibitors to restrict further the potential for viral escape, possibly by forcing losses in fitness from having to elude multiple inhibitors. Although patients are well served at present, it is clear that new ideas and new directions are still viable and ripe for consideration as worthy investments.

References

1. UNAIDS, United Nations Program on HIV/AIDS (UNAIDS), *UNAIDS Report on the Global AIDS Epidemic*, December 2012, www.UNAIDS.org (last accessed 23 February 2013).
2. A. Maitland, A. Jackson, J. Osorio, S. Mandalia, B. G. Gazzard and G. J. Moyle, *HIV Med.*, 2008, **9**, 667.
3. J. B. Nachega, V. C. Marconi, G. U. van Zyl, E. M. Gardner, W. Preiser, S. Y. Hong, E. J. Mills and R. Gross, *Infect. Disord. Drug Targets*, 2011, **11**, 167.
4. A. S. Fauci and D. M. Morens, *N. Engl. J. Med.*, 2012, **366**, 454.
5. H.-H. M. Truong, T. A. Kellogg, W. McFarland, B. Louie, J. D. Klausner, S. S. Philip and R. M. Grant, *Plos One*, 2011, **6**, 1.
6. L. Wittkop, H. F. Günthard, D. Dunn, A. Cossi-Lepri, A. de Luca, C. Kücherer, N. Obel, V. von Wyl, B. Masquelier, C. Stephan, C. Torti, A. Antinori, F. Garcia, A. Judd, K. Porter, R. Thiébaut, H. Castro, A. van Sighem, C. Colin, J. Kjaer, J. D. Lundgren, R. Paredes, A. Pozniak, B. Clotet, A. Philips, D. Pillay and G. Chêne, *Lancet Infect. Dis.*, 2011, **11**, 363.
7. V. Jain, T. Liegler, E. Vittinghoff, W. Hartogensis, P. Bacchetti, L. Poole, L. Loeb, C. D. Pilcher, R. M. Grant, S. G. Deeks and F. M. Hecht, *Plos One*, 2010, **5**, 1.
8. Panel on Antiretroviral Guidelines for Adults and Adolescents, Department of Health and Human Services, *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents*, 2011, <http://aidsinfo.nih.gov/contentfiles/adultandadolescentGL.pdf>.

9. B. Taiwo, L. Zheng, S. Gallien, R. M. Matining, D. R. Kuritzkes, C. C. Wilson, B. I. Berzins, E. P. Acosta, B. Bastow, P. S. Kim and J. J. Eron Jr., *AIDS*, 2011, **25**, 2113.
10. J. Reynes, A. Lawal, F. Pulido, R. Soto-Malave, J. Gathe, M. Tian, L. M. Frerick, T. J. Podsadecki and A. M. Nilius, *HIV Clin. Trials*, 2011, **12**, 255.
11. A. S. Perelson and S. G. Deeks, *Sci. Transl. Med.*, 2011, **3**, 91ps30.
12. A. D. Frankel and J. A. T. Young, *Annu. Rev. Biochem.*, 1998, **67**, 1.
13. P. Rice, R. Craigie and D. R. Davies, *Curr. Opin. Struct. Biol.*, 1996, **6**, 76.
14. F. Dyda, A. B. Hickman, T. M. Jenkins, A. Engelman, R. Craigie and D. R. Davies, *Science*, 1994, **266**, 1981.
15. L. Haren, B. Ton-Hoang and M. Chandler, *Annu. Rev. Microbiol.*, 1999, **53**, 245.
16. J. A. Grobler, K. Stillmock, B. Hu, M. Witmer, P. Felock, A. S. Espeseth, A. Wolfe, M. Egbertson, M. Bourgeois, J. Melamed, J. S. Wai, S. Young, J. Vacca and D. J. Hazuda, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 6661.
17. J. S. Wai, M. S. Egbertson, L. S. Payne, T. E. Fisher, M. W. Embrey, L. O. Tran, J. Y. Melamed, H. M. Langford, J. P. Guare Jr., L. Zhuang, V. E. Grey, J. P. Vacca, M. K. Holloway, A. M. Naylor-Olsen, D. J. Hazuda, P. J. Felock, A. L. Wolfe, K. A. Stillmock, W. A. Schleif, L. J. Gabryelski and S. D. Young, *J. Med. Chem.*, 2000, **43**, 4923.
18. T. Fujishita and T. Yoshinaga, *PCT Int. Appl.*, WO1999/50245, 1999; T. Fujishita, T. Yoshinaga and A. Sato, *PCT Int. Appl.*, WO2000/3, 1999, 9086.
19. R. Kiyama and T. Kawasuji, *PCT Int. Appl.*, WO01/95905, 2001.
20. T. Kawasuji, M. Fuji, T. Yoshinaga, A. Sato, T. Fujiwara and R. Kiyama, *Bioorg. Med. Chem.*, 2006, **14**, 8420.
21. T. Kawasuji, T. Yoshinaga, A. Sato, M. Yodo, T. Fujiwara and R. Kiyama, *Bioorg. Med. Chem.*, 2006, **14**, 8430.
22. T. Kawasuji, M. Fuji, T. Yoshinaga, A. Sato, T. Fujiwara and R. Kiyama, *Bioorg. Med. Chem.*, 2007, **15**, 5487; M. J. C. Rosemond, L. John-Williams, T. Yamaguchi, T. Fujishita and J. S. Walsh, *Chem.-Biol. Interact.*, 2004, **147**, 129.
23. D. J. Hazuda, P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J. A. Grobler, A. Espeseth, L. Gabryelski, W. Schleif, C. Blau and M. D. Miller, *Science*, 2000, **287**, 646.
24. A. S. Espeseth, P. Felock, A. Wolfe, M. Witmer, J. A. Grobler, N. Anthony, M. Egbertson, J. Y. Melamed, S. Young, T. Hamill, J. L. Cole and D. J. Hazuda, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 11244.
25. L. Krishnan, X. Li, H. L. Naraharisetty, S. Hare, P. Cherepanov and A. Engelman, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 15910.
26. S. Hare, S. S. Gupta, E. Valkov, A. Engelman and P. Cherepanov, *Nature*, 2010, **464**, 232.
27. S. Hare, A. M. Vos, R. F. Clayton, J. W. Thuring, M. D. Cummings and P. Cherepanov, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 20057.

28. S. Chung, D. M. Himmel, J. -K. Jiang, K. Wojtak, J. D. Bauman, J. W. Rausch, J. A. Wilson, J. A. Beutler, C. J. Thomas, E. Arnold and S. F. J. Le Grice, *J. Med. Chem.*, 2011, **54**, 4462.
29. E. B. Lansdon, Q. Liu, S. A. Leavitt, M. Balakrishnan, J. K. Perry, C. Lancaster-Moyer, N. Kutty, X. Liu, N. H. Squires, W. J. Watkins and T. A. Kirschberg, *Antimicrob. Agents Chemother.*, 2011, **55**, 2905.
30. K. E. B. Parkes, P. Ermert, J. Fassler, J. Ives, J. A. Martin, J. H. Merrett, D. Obrecht, G. Williams and K. Klumpp, *J. Med. Chem.*, 2003, **46**, 1153.
31. A. Pendri, N. A. Meanwell, K. M. Peese and M. A. Walker, *Expert Opin. Ther. Pat.*, 2011, **21**, 1173.
32. B. A. Johns and A. C. Svolto, *Expert Opin. Ther. Pat.*, 2008, **18**, 1225.
33. B. A. Johns, *Annu. Rep. Med. Chem.*, 2010, **45**, 263.
34. S. Little, G. Drusano, R. Schooley, D. Haas, P. Kumar, S. Hammer, D. McMahon, K. Squires, R. Asfour, D. Richman, J. Chen, A. Saah, R. Leavitt, D. Hazuda and B. Y. Nguyen, presented at the 12th Conference on Retroviruses and Opportunistic Infections, Boston, MA, February 2005, Abstract 161.
35. E. P. Garvey, B. A. Johns, M. J. Gartland, S. A. Foster, W. H. Miller, R. G. Ferris, R. J. Hazen, M. R. Underwood, E. E. Boros, J. B. Thompson, J. G. Weatherhead, C. K. Koble, S. H. Allen, L. T. Schaller, R. G. Sherrill, T. Yoshinaga, M. Kobayashi, C. Wakasa-Morimoto, S. Miki, K. Nakahara, T. Noshi, A. Sato and T. Fujiwara, *Antimicrob. Agents Chemother.*, 2008, **52**, 901.
36. G. S. Jones, F. Yu, A. Zeynalzadegan, J. Hesselgesser, X. Chen, J. Chen, H. Jin, C. U. Kim, M. Wright, R. Geleziunas and M. Tsiang, *Antimicrob. Agents Chemother.*, 2009, **53**, 1194.
37. Z. Lin, D. Langley, B. Terry, T. Protack, M. A. Walker, B. N. Naidu, M. Patel, N. Meanwell, M. Krystal and I. B. Dicker, presented at the 19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 5-8 March 2012, Abstract 690.
38. M. Rowley, *Prog. Med. Chem.*, 2008, **46**, 1.
39. V. Summa, A. Petrocchi, F. Bonelli, B. Crescenzi, M. Donghi, M. Ferrara, F. Fiore, C. Gardelli, O. G. Paz, D. J. Hazuda, P. Jones, O. Kinzel, R. Laufer, E. Monteagudo, E. Muraglia, E. Nizi, F. Orvieto, P. Pace, G. Pescatore, R. Scarpelli, K. Stillmock, M.V. Witmer and M. Rowley, *J. Med. Chem.*, 2008, **51**, 5843.
40. M. A. Thompson, J. A. Adberg, P. Cahn, J. S. G. Montaner, G. Rizzardini, A. Telenti, J. M. Gatell, H. F. Günthard, S. M. Hammer, M. S. Hirsch, D. M. Jacobsen, P. Reiss, D. D. Richman, P. A. Volberding, P. Yeni and R. T. Schooley, *JAMA*, 2010, **304**, 321.
41. A. Zolopa, *Antiviral Res.*, 2010, **85**, 241.
42. J. M. Murray, S. Emery, A. D. Kelleher, M. Law, J. Chen, D. J. Hazuda, B.-Y. T. Nguyen, H. Teppler and D. A. Cooper, *AIDS*, 2007, **21**, 2315.
43. R. A. Steigbigel, D. A. Cooper, P. N. Kumar, J. E. Enron, M. Schechter, M. Markowitz, M. R. Loutfy, J. L. Lennox, J. M. Gatell,

- J. K. Rockstroh, C. Katlama, P. Yeni, A. Lazzarin, B. Clotet, J. Zhao, J. Chen, D. M. Ryan, R. R. Rhodes, J. A. Killar, L. R. Gilde, K. M. Strohmaier, A. R. Meibohm, M. D. Miller, D. J. Hazuda, M. L. Nessly, M. J. DiNubile, R. D. Isaacs, B.-Y. Nguyen and H. Teppler, *N. Engl. J. Med.*, 2008, **359**, 339.
44. R. A. Steigbigel, D. A. Cooper, H. Teppler, J. E. Enron, P. N. Kumar, J. K. Rockstroh, M. Schecter, C. Katlama, M. Markowitz, P. Yeni, M. R. Loutfy, A. Lazzarin, J. L. Lennox, B. Clotet, J. Zhao, H. Wan, R. R. Rhodes, K. M. Strohmaier, R. J. Barnard, R. D. Isaacs and B.-Y. Nguyen, *Clin. Infect. Dis.*, 2010, **50**, 605.
45. D. A. Cooper, R. A. Steigbigel, J. M. Gatell, J. K. Rockstroh, C. Katlama, P. Yeni, A. Lazzarin, B. Clotet, P. N. Kumar, J. E. Enron, M. Schecter, M. Markowitz, M. R. Loutfy, J. L. Lennox, J. Zhao, J. Chen, D. M. Ryan, R. R. Rhodes, J. A. Killar, L. R. Gilde, K. M. Strohmaier, A. R. Meibohm, M. D. Miller, D. J. Hazuda, M. L. Nessly, M. J. DiNubile, R. D. Isaacs and H. Teppler, *N. Engl. J. Med.*, 2008, **359**, 355.
46. D. da Silva, L. Van Wesenbeeck, D. Breilh, S. Reigadas, G. Anies, K. Van Baelen, P. Morlat, D. Neau, M. Dupon, L. Wittkop, H. Fleury and B. Masquelier, *J. Antimicrob. Chemother.*, 2010, **65**, 1262.
47. O. Delelis, S. Thierry, F. Subra, F. Simon, I. Malet, C. Alloui, S. Sayon, V. Calvez, E. Deprez, A.-G. Marcelin, L. Tchertanov and J.-F. Mouscadet, *Antimicrob. Agents Chemother.*, 2010, **54**, 491.
48. M. Métifiot, C. Marchand, K. Maddali and Y. Pommier, *Viruses*, 2010, **2**, 1347.
49. M. Métifiot, K. Maddali, A. Naumova, X. Zhang, C. Marchand and Y. Pommier, *Biochemistry*, 2010, **49**, 3715.
50. J. L. Lennox, E. DeJesus, A. Lazzarin, R. B. Pollard, J. V. R. Madruga, D. S. Berger, J. Zhao, X. Xu, A. Williams-Diaz, A. J. Rodgers, R. J. O. Barnard, R. M. D. Miller, M. J. DiNubile, B.-Y. Nguyen, R. Leavitt and P. Sklar, *Lancet*, 2009, **374**, 796.
51. J. L. Lennox, E. DeJesus, D. S. Berger, A. Lazzarin, R. B. Pollard, J. V. R. Madruga, J. Zhao, H. Wan, C. L. Gilbert, H. Teppler, A. J. Rodgers, R. J. O. Barnard, M. D. Miller, M. J. DiNubile, B.-Y. Nguyen, R. Leavitt and P. Sklar, *J. Acquir. Immune Defic. Syndr.*, 2010, **55**, 39.
52. J. J. Eron, B. Young, D. A. Cooper, M. Youle, E. DeJesus, J. Andrade-Villaneuva, C. Workman, R. Zajdenverg, G. Fätkenheuer, D. S. Berger, P. N. Kumar, A. J. Rodgers, M. A. Shaughnessy, M. L. Walker, R. J. O. Barnard, M. D. Miller, M. J. DiNubile, B.-Y. Nguyen, R. Leavitt, X. Xu and P. Sklar, *Lancet*, 2010, **375**, 396.
53. E. Martinez, M. Larrousse, J. M. Llibre, F. Gutierrez, M. Saumoy, A. Antela, H. Knobel, J. Murillas, J. Berenguer, J. Pich, I. Perez and J. Gatell, *AIDS*, 2010, **24**, 1697.
54. M. A. Wainberg, G. J. Zaharatos and B. G. Brenner, *N. Engl. J. Med.*, 2011, **365**, 637.

55. J. J. Eron, J. K. Rockstroh, J. Reynes, J. Andrade-Villanueva, J. V. Ramalho-Madruga, L.-G. Bekker, B. Young, C. Katlama, J. M. Gatell-Artigas, J. R. Arribas, M. Nelson, H. Campbell, J. Zhao, A. J. Rodgers, M. L. Rizk, L. Wenning, M. D. Miller, D. J. Hazuda, M. J. DiNubile, R. Leavitt, R. Isaacs, M. N. Robertson, P. Sklar and B.-Y. Nguyen, *Lancet Infect. Dis.*, 2011, **11**, 907.
56. M. Iwamoto, L. A. Wenning, A. S. Petry, M. Laethem, M. De Smet, J. T. Kost, S. A. Merschman, K. M. Strohmaier, S. Ramael, K. C. Lasseter, J. A. Stone, K. M. Gottesdiener and J. A. Wagner, *Clin. Pharm. Ther.*, 2007, **83**, 293.
57. M. Markowitz, J. O. Morales-Ramirez, B.-Y. Nguyen, C. M. Kovacs, R. A. Steigbigel, D. A. Cooper, R. Liporace, R. Schwartz, R. Isaacs, L. R. Gilde, L. Wenning, J. Zhao and H. Teppler, *J. Acquir. Immune Defic. Syndr.*, 2006, **43**, 509.
58. M. Sato, T. Motomura, H. Aramaki, T. Matsuda, M. Yamashita, Y. Ito, H. Kawakami, Y. Matsuzaki, W. Watanabe, K. Yamataka, S. Ikeda, E. Kodama, M. Matuoka and H. Shinkai, *J. Med. Chem.*, 2006, **49**, 1506.
59. M. Sato, H. Kawakami, T. Motomura, H. Aramaki, T. Matsuda, M. Yamashita, Y. Ito, Y. Matsuzaki, K. Yamataka, S. Ikeda and H. Shinkai, *J. Med. Chem.*, 2009, **52**, 4869.
60. Y. Matsuzaki, W. Watanabe and K. Yamataka, presented at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO, 5–9 February 2006, Abstract 508.
61. T. Willis and V. Vega, *Expert Opin. Invest. Drugs*, 2012, **21**, 395.
62. K. Shimura and E. N. Kodama, *Antiviral Chem. Chemother.*, 2012, **20**, 79.
63. E. DeJesus, D. Berger, M. Markowitz, C. Cohen, T. Hawkins, P. Ruane, R. Elion, C. Farthing, L. Zhong, A. K. Cheng, D. McColl and B. P. Kearney, *J. Acquir. Immune Defic. Syndr.*, 2006, **43**, 1.
64. S. Ramanathan, A. A. Mathias, P. German and B. P. Kearney, *Clin. Pharmacokinet.*, 2011, **50**, 229.
65. S. Ramanathan, G. Shen, A. Cheng and B. P. Kearney, *J. Acquir. Immune Defic. Syndr.*, 2007, **45**, 274.
66. A. A. Mathias, P. German, B. P. Murray, L. Wei, A. Jain, S. West, D. Warren, J. Hui and B. P. Kearney, *Clin. Pharmacol. Ther.*, 2010, **87**, 322.
67. P. German, D. Warren, S. West, J. Hui and B. P. Kearney, *J. Acquir. Immune Defic. Syndr.*, 2010, **55**, 323.
68. C. Marchand, *Expert Opin. Investig. Drugs*, 2012, **21**, 901.
69. A. R. Zolopa, D. S. Berger, H. Lampiris, L. Zhong, S. L. Chuck, J. V. Enejosa, B. P. Kearney and A. K. Cheng, *J. Infect. Dis.*, 2010, **201**, 814.
70. C. Cohen, R. Elion, P. Ruane, D. Shamblaw, E. DeJesus, B. Rashbaum, S. L. Chuck, K. Yale, H. C. Liu, D. R. Warren, S. Ramanathan and B. P. Kearney, *AIDS*, 2011, **25**, F7.
71. J.-M. Molina, A. LaMarca, J. Andrade-Villanueva, B. Clotet, N. Clumeck, Y.-P. Liu, L. Zhong, N. Margot, A. K. Cheng and S. L. Chuck, *Lancet Infect. Dis.*, 2012, **12**, 27.

72. P. Sax, E. DeJesus, A. Mills, A. Zolopa, C. Cohen, D. Wohl, J. Gallant, H. Liu, E. Quirk and B. P. Kearney, presented at the 19th Conference of Retroviruses and Opportunistic Infections, Seattle, WA, 5–8 March 2012, Abstract 101.
73. P. E. Sax, E. DeJesus, A. Mills, A. Zolopa, C. Cohen, D. Wohl, J. E. Gallant, H. C. Liu, L. Zhong, K. Yale, K. White, B. P. Kearney, J. Szwarcberg, E. Quirk and A. K. Cheng, *Lancet*, 2012, **379**, 2439.
74. E. DeJesus, J. Rockstroh, J.-M. Molina, J. Gathe, S. Ramanathan, X. Wei, J. Szwarcberg, A. Jandourek and A. Cheng, presented at the 19th Conference of Retroviruses and Opportunistic Infections, Seattle, WA, 5–8 March 2012, Abstract 627.
75. E. DeJesus, J. K. Rockstroh, J. –M. Molina, J. Gathe, S. Ramanathan, X. Wei, K. Yale, J. Szwarcberg, K. White, A. K. Cheng and B. P. Kearney, *Lancet*, 2012, **379**, 2429.
76. O. Goethals, R. Clayton, M. Van Ginderen, I. Vereycken, E. Wagemans, P. Geluykens, K. Dock, R. Strijbos, V. Smits, A. Vos, G. Meersseman, D. Jochmans, K. Vermeire, D. Schols, A. Hallenberger and K. Hertogs, *J. Virol.*, 2008, **82**, 10366.
77. K. Shimura, E. Kodama, Y. Sakagami, Y. Matsuzaki, W. Watanabe, K. Yamataka, Y. Watanabe, Y. Ohata, S. Doi, M. Sato, M. Kano, S. Ikeda and M. Matuoka, *J. Virol.*, 2008, **82**, 764.
78. A. Hombrouch, A. Voet, B. Van Remoortel, C. Desadeleer, M. De Maeyer, Z. Debyser and M. Witvrouw, *Antimicrob. Agents Chemother.*, 2008, **52**, 2069.
79. J. Marinello, C. Marchand, B. T. Mott, A. Bain, C. J. Thomas and Y. Pommier, *Biochemistry*, 2008, **47**, 9345.
80. J.-L. Blanco, V. Varghese, S.-Y. Rhee, J. M. Gatell and R. W. Shafer, *J. Infect. Dis.*, 2011, **203**, 1204.
81. B. Taiwo, L. Zheng, S. Gallien, R. M. Matining, D. R. Kuritzkes, C. C. Wilson, B. I. Berzins, E. P. Acosta, B. Bastow, P. S. Kim and J. J. Eron, *AIDS*, 2011, **25**, 2113.
82. M. Métifiot, N. Vandegraaff, K. Maddali, A. Naumova, X. Zhang, D. Rhodes, C. Marchand and Y. Pommier, *AIDS*, 2011, **25**, 1175.
83. P. K. Quashie, R. D. Sloan and M. A. Wainberg, *BMC Med.*, 2012, **10**, 34.
84. R. Collier, *CMAJ*, 2009, **180**, 279.
85. E. Serrao, S. Odde, K. Ramkumar and N. Neamati, *Retrovirology*, 2009, **6**, 25.
86. H. Murai, T. Endo, N. Kurose, T. Taishi and H. Yoshida, *PCT Int. Appl.*, WO04/024693, 2004.
87. M. Egbertson, H. M. Moritz, J. Y. Melamed, W. Han, D. S. Perlow, M. S. Kuo, M. Embrey, J. P. Vacca, M. M. Zrada, A. R. Cortes, A. Wallace, Y. Leonard, D. J. Hazuda, M. D. Miller, P. J. Felock, K. A. Stillmock, M. V. Witmer, W. Schlieff, L. J. Gabryelski, G. Moyer, J. D. Ellis, L. Jin, W. Xu, M. P. Braun, K. Kassahun, N. N. Tsou and S. D. Young, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1392.
88. E. E. Boros, C. E. Edwards, S. A. Foster, M. Fuji, T. Fujiwara, E. P. Garvey, P. L. Golden, R. J. Hazen, J. L. Jeffrey, B. A. Johns,

- T. Kawasuji, R. Kiyama, C. S. Koble, N. Kurose, W. H. Miller, A. L. Mote, H. Murai, A. Sato, J. B. Thompson, M. C. Woodward and T. Yoshinaga, *J. Med. Chem.*, 2009, **52**, 2754.
89. M. Egbertson, J. Y. Melamed, H. M. Langford and S. D. Young, *PCT Int. Appl.*, WO03/062204, 2003.
 90. T. Kawasuji, B. Johns, H. Yoshida, T. Taishi, Y. Taoda, H. Murai, R. Kiyama, M. Fuji, T. Yoshinaga, T. Seki, M. Kobayashi, A. Sato and T. Fujiwara, *J. Med. Chem.*, 2012, **55**, 8735.
 91. G. Jarmy, M. Heinelein, B. Weissbrich, C. Jassoy and A. Rethwilm, *J. Med. Virol.*, 2001, **64**, 223.
 92. B. Johns, T. Kawasuji, T. Taishi, H. Yoshida, E. Garvey, W. Spreen, M. Underwood, A. Sato, T. Yoshinaga and T. Fujiwara, presented at the 17th Conference of Retroviruses and Opportunistic Infections, San Francisco, CA, 16–19 February 2010, Abstract 55.
 93. M. Kobayashi, T. Yoshinaga, T. Seki, C. Wakasa-Morimoto, K. W. Brown, R. Ferris, S. A. Foster, R. J. Hazen, S. Miki, A. Suyama-Kagitani, S. Kawauchi-Miki, T. Taishi, T. Kawasuji, B. A. Johns, M. R. Underwood, E. P. Garvey, A. Sato and T. Fujiwara, *Antimicrob. Agents Chemother.*, 2011, **55**, 813.
 94. B. A. Johns, T. Kawasuji, J. G. Weatherhead, T. Taishi, D. P. Temelkoff, H. Yoshida, T. Akiyama, Y. Taoda, H. Murai, R. Kiyama, M. Fuji, N. Tanimoto, J. Jeffrey, S. A. Foster, T. Yoshinaga, T. Seki, M. Kobayashi, A. Sato, M. N. Johnson, E. P. Garvey and T. Fujiwara, *J. Med. Chem.*, submitted for publication.
 95. B. Davies and T. Morris, *Pharm. Res.*, 1993, **10**, 1093.
 96. S. Min, I. Song, J. Borland, S. Chen, Y. Lou, T. Fujiwara and S. Piscitelli, *Antimicrob. Agents Chemother.*, 2010, **54**, 254.
 97. S. Min, L. Sloan, E. DeJesus, T. Hawkins, L. McCurdy, I. Song, R. Stroder, S. Chen, M. Underwood, T. Fujiwara, S. Piscitelli and J. Lalezari, *AIDS*, 2011, **25**, 1737.
 98. I. Song, S. Min, J. Borland, Y. Lou, S. Chen, P. Patel, T. Ishibashi and S. Piscitelli, *J. Clin. Pharmacol.*, 2011, **51**, 237.
 99. I. Song, J. Borland, S. Chen, Y. Lou, A. Peppercorn, T. Wajima, S. Min and S. Piscitelli, *Br. J. Clin. Pharmacol.*, 2011, **72**, 103.
 100. I. Song, S. Min, J. Borland, Y. Lou, S. Chen, T. Ishibashi, T. Wajima and S. Piscitelli, *J. Acquir. Immune Defic. Syndr.*, 2010, **55**, 365.
 101. P. Patel, I. Song, J. Borland, A. Patel, Y. Lou, S. Chen, T. Wajima, A. Peppercorn, S. S. Min and S. C. Piscitelli, *J. Antimicrob. Chemother.*, 2011, **66**, 1567.
 102. I. Song, J. Borland, S. Chen, P. Patel, T. Wajima, A. Peppercorn and S. C. Piscitelli, *Antimicrob. Agents Chemother.*, 2012, **56**, 1627.
 103. I. Song, J. Borland, S. Min, Y. Lou, S. Chen, P. Patel, T. Wajima and S. C. Piscitelli, *Antimicrob. Agents Chemother.*, 2011, **55**, 3517.
 104. J. Van Lunzen, F. Maggiolo, J. R. Arribas, A. Rakhmanova, P. Yeni, B. Young, J. K. Rockstroh, S. Almond, I. Song, C. Brothers and S. Min, *Lancet Infect. Dis.*, 2012, **12**, 111.

105. H.-J. Stellbrink, J. Reynes, A. Lazzarin, E. Voronin, F. Pulido, F. Felizarta, S. Almond, M. St. Clair, N. Flack and S. Min, presented at the 19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 5–8 March 2012, Abstract K-102.
106. V. Soriano, J. Cox, J. Eron, P. Kumar, C. Katlama, A. Lazzarin, I. Poizot-Martin, G. Richmond, M. Ait-Khaled, T. Fujiwara, J. Huang, S. Min, C. Vavro and J. M. Yeo, presented at the 13th European AIDS Conference, Belgrade, Serbia, 12–15 October 2011.
107. J. Eron, P. Kumar, A. Lazzarin, G. Richmond, V. Soriano, J. Huang, C. Vavro, M. Ait-Khaled, S. Min and J. Yeo, presented at the 18th Conference on Retroviruses and Opportunistic Infections, Boston, MA, 27 February–2 March 2011.
108. F. Raffi, A. Rachlis, H.-J. Stellbrink, W. D. Hardy, C. Torti, C. Orkin, M. Bloch, D. Podzamczar, V. Pokrovsky, S. Almond, D. Margolis and S. Min, presented at the XIX International AIDS Conference, Washington, DC, 22–27 July 2012, Abstract THLB04.
109. S. Walmsley, A. Antela, N. Clumeck, D. Duiculescu, A. Eberhard, F. Gutiérrez, L. Hocqueloux, F. Maggiolo, U. Sandkovsky, C. Granier, B. Wynne and K. Pappa, presented at the 52nd Interscience Conference on Antimicrobials and Chemotherapy (ICAAC), San Francisco, CA, 9–12 September 2012, Abstract H-556b.
110. M. R. Underwood, M. St. Clair, B. A. Johns, A. Sato, T. Fujiwara and W. Spreen, presented at the XVIII International AIDS Conference, Vienna, 18–23 July 2010.
111. S. Min, E. DeJesus, L. McCurdy, G. Richmond, J. Torres, S. Ford, S. Chen, Y. Lou, M. Bomar, T. Cyr, M. St.Clair, T. Fujiwara and S. Piscitelli, presented at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, CA, 12–15 September 2009, paper H-1228.
112. S. L. Ford, S. Chen, E. Gould, E. Dumont, Y. Lou, S. Piscitelli, T. Taishi and W. Spreen, presented at the 13th International Workshop on Clinical Pharmacology of HIV Therapy, Barcelona, 2012, paper P-15.
113. W. Spreen, S. L. Ford, S. Chen, E. Gould, D. Wilfret, D. Subich, T. Taishi and Z. Hong, presented at the XIX International AIDS Conference, Washington, DC, 22–27 July 2012, Abstract TUPE040.
114. M. Llano, M. Vanegas, N. Hutchins, D. Thompson, S. Delgado and E. M. Poeschla, *J. Mol Biol.*, 2006, **360**, 760.
115. P. Cherepanov, E. Devroe, P. A. Silver and A. Engelman, *J. Biol. Chem.*, 2004, **279**, 48883.
116. M. Vanegas, M. Llano, S. Delago, D. Thompson, M. Peretz and E. Poeschla, *J. Cell Sci.*, 2005, **118**, 1733.
117. M. Llano, M. Vanegas, O. Fregoso, D. Saenz, S. Chung, M. Peretz and E. M. Poeschla, *J. Virol.*, 2004, **78**, 9524.
118. A. Engelman and P. Cherepanov, *PLoS Pathogen*, 2008, **4**, e1000046.
119. S. Emiliani, A. Mousnier, K. Busschots, M. Maroun, B. Van Maele, D. Tempe, L. Vandekerckhove, F. Moisant, L. Ben-Slama, M. Witvrouw,

- F. Christ, J.-C. Rain, C. Dargemont, Z. Debyser and R. Benarous, *J. Biol. Chem.*, 2005, **280**, 25517.
120. L. Vandekerckhove, F. Christ, B. Van Maele, J. De Rijck, R. Gijssbers, C. Van den Haute, M. Witvrouw and Z. Debyser, *J. Virol.*, 2006, **80**, 1886.
 121. G. Maertens, P. Cherepanov, W. Pluymers, K. Busschots, E. De Clercq, Z. Debyser and Y. Engelborghs, *J. Biol. Chem.*, 2003, **278**, 33528.
 122. G. Maertens, P. Cherepanov, Z. Debyser, Y. Engelborghs and A. Engelman, *J. Biol. Chem.*, 2004, **279**, 33421.
 123. G. Yu, G. S. Jones, M. Hung, A. H. Wagner, H. L. MacArthur, X. Liu, S. Leavitt, M. J. McDermott and M. Tsiang, *Biochemistry*, 2007, **46**, 2899.
 124. M. Llano, S. Delgado, M. Vanegas and E. M. Poeschla, *J. Biol. Chem.*, 2004, **279**, 55570.
 125. P. Cherepanov, A. L. B. Ambrosio, S. Rahman, T. Ellenberger and A. Engelman, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 17309.
 126. C. J. McKee, J. J. Kessl, N. Shkriabai, M. J. Dar, A. Engelman and M. Kvaratskeli, *J. Biol. Chem.*, 2008, **283**, 31802.
 127. F. Christ, A. Voet, A. Marchand, S. Nicolet, B. A. Desimmie, D. Marchand, D. Bardiot, N. J. Van der Veken, B. Van Remoortel, S. V. Strelkov, M. De Maeyer, P. I. Chaltin and Z. Debyser, *Nat. Chem. Biol.*, 2010, **6**, 442.
 128. Y. S. Tsantrizos, M. Boes, C. Brochu, C. Fenwick, E. Malenfant, S. Mason and M. Pesant, *PCT Int. Appl.*, 2007, WO2007/131350A1.
 129. C. Yoakim, M. Amad, M. D. Bailey, R. Bethell, M. Bös, P. Bonneau, M. Cordingley, R. Coulombe, J. Duan, P. Edwards, L. Fader, A.-M. Faucher, M. Garneau, A. Jakalian, S. Kawai, L. Lamorte, S. LaPlante, L. Luo, S. Mason, M.-A. Poupart, N. Rioux, B. Simoneau, Y. Tsantrizos, M. Witvrouw and C. Fenwick, presented at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, IL, 17–20 September 2011, Abstract F1–1369.
 130. C. Fenwick, R. Bethell, A. Quinson, P. Robinson, B. Simoneau and C. Yoakim, presented at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, IL, 17–20 September 2011, Abstract F1–1370.
 131. S. Aslanyan, C. Ballow, J. P. Sabo, J. Halbeck, D. Roos, T. R. Macgregor, P. Robinson and J. Kort, presented at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, IL, 17–20 September 2011, Abstract AI-1725.
 132. J. J. Kessl, N. Jena, Y. Koh, S. –H. Taskent, A. Slaughter, L. Feng, S. DeSilva, L. Wu, S. F. Le Grice, A. Engelman, J. R. Fuchs and M. Kvaratskhelia, *J. Biol. Chem.*, 2012, **287**, 16801.
 133. Z. Hayouka, J. Rosenbluh, A. Levin, S. Loya, M. Lebendiker, D. Veprintsev, M. Kotler, A. Hizi, A. Loyter and A. Friedler, *Proc. Natl. Acad. Sci., U. S. A.*, 2007, **104**, 8316.